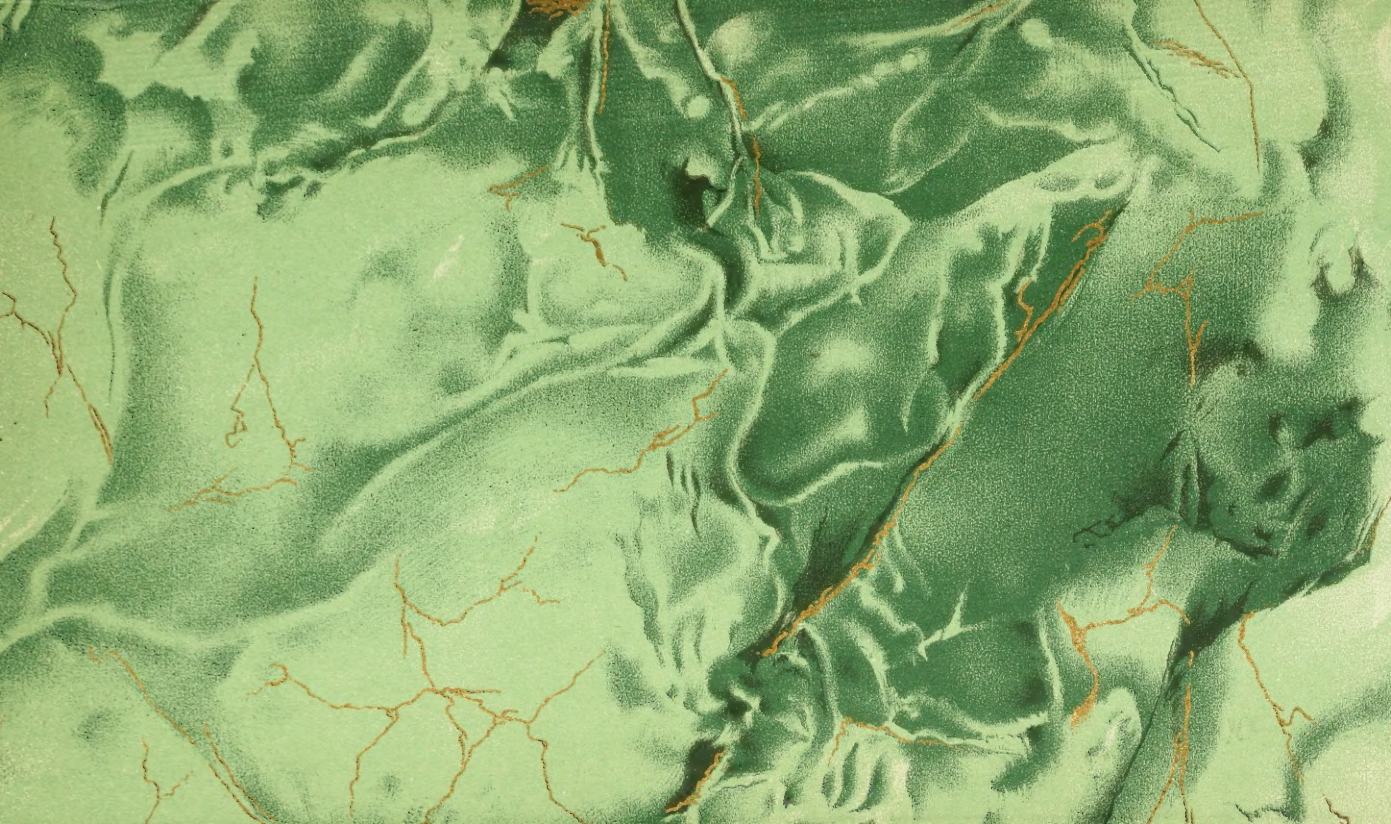

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CONTENTS

RESEARCH INVESTIGATIONS

ON THE ASSUMED DESTRUCTION OF TRYPSIN BY PEPSIN AND ACID

II. OBSERVATIONS ON ANIMALS *

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(From the Journal of the American Chemical Society, January, 1917)

In our recent paper with the above title[1] we showed by a long series of experiments in vitro that the common proteolytic enzyme of the pancreas, isolated as trypsin, is capable of withstanding a rather long digestion in presence of hydrochloric acid and pepsin, provided sufficient protein of some form is present to combine with all or part of the acid and so bring the hydrogen-ion concentration down to a certain level. This behavior of protein is of great practical importance, and failure to recognize it led to much confusion in the study of the mutual action of certain enzymes. It was further brought out that trypsin must exhibit a certain degree of activity in solutions which have a faintly acid reaction, rather than the usual alkaline reaction. This is contrary to the long accepted notion that this enzyme is active in alkaline medium only, but many phenomena point to this relation.

In the subsequent prosecution of the work we have relied on evidence secured through experiments on

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

1. Jour. Am. Chem. Soc., 1916, **38**, 1620.

animals, partly with dogs and partly with the human subject. Satisfactory methods were not immediately available. In the work with dogs our first attempts were in this direction. Duodenal fistulas were made in a number of animals at a point between the pylorus and the entrance of the main pancreatic duct. A bent glass canula introduced into this opening was provided with an expanded end and so turned that it would collect the chyme flow from the stomach, but would prevent the upward flow of the bile and pancreatic secretion. Working in this way it appeared possible to obtain the unmixed gastric contents. The dogs so treated were fed on prepared foods to which the desired amount of trypsin was added. After remaining in the stomach through a sufficient interval the partially digested mixtures were allowed to flow through the cannula to be collected and examined for the persisting trypsin. But unfortunately no data of positive value could be obtained by this general scheme, because, as a rule, the animals could not be kept alive long enough to give results which might be considered normal. This theoretically simple plan was tried in consequence of the desire to eliminate at the start any complication arising from the presence of pancreatic trypsin, but the physiological difficulties in the way of securing a permanent fistula in the short space between the bile duct and the pylorus seem to be very great.[2]

There is this practical objection to the introduction of a duodenal fistula at a much lower point, along with the section or ligature of the pancreatic duct. The chyme secured in this way contains too much admixed bile, and besides there is always the possibility of the presence of erepsin to obscure the interpretation of the results of the tests on the chyme for trypsin.

As the prime object of the investigation was to determine the presence or absence of tryptic activity in mixtures of pepsin, acid and food, as such mix-

2. Our thanks are due to Dr. W. R. Cubbins for his kindness in making a number of duodenal fistulas for us in our efforts to utilize the method.

tures may exist in the stomach, it was finally decided to make the observations on the stomach itself, and in such a manner as to exclude or to allow for the interference from the regurgitated intestinal contents which might contain, possibly, some trypsin. To carry out a plan of this sort several methods of observation are available. We have begun by feeding certain definite test meals to dogs and after a time withdrawing the stomach contents as far as possible and examining for acidity, presence of bile as suggesting regurgitation, and peptic and tryptic activity. This was done first with the normal diet and was followed by a similar meal to which a known weight of active trypsin was added. The problem was to determine the existence of increased tryptic activity after the ingestion of the commercial enzyme.

EXPERIMENTS ON THE NORMAL STOMACH

The trypsin preparations used in the tests were the same as described in our last paper, and had the strength there given. The usual test meal consisted of 16 gm. of cracker crumbs with 200 c.c. of water, to which 50 or 100 gm. of cooked and finely ground meat were added. To insure the ingestion of the whole of the food, or food and trypsin, the mixtures were fed through a tube and funnel and washed down with the last of the water. Occasionally some egg was added to the meal and the water was at times increased. After a lapse of forty-five to 120 minutes from the time of feeding, the tube was introduced and the remaining contents drawn off as rapidly as possible. The volume of the liquid collected was noted. A small fraction of this was used for tests for free and combined acid, and separate portions for pepsin and bile tests. It may be said here that in all cases examined a good pepsin reaction was obtained, which fact need not be mentioned in the tables. Bile was usually absent, and in such cases, when no trypsin was ingested with the food, a tryptic reaction was practically negative, or so slight as to be questionable. With bile present from regurgitation a trypsin test was usualy positive, but weak.

The trypsin test was made on a larger aliquot part of the stomach contents and in this manner: Twenty-five c.c. (occasionally less) of the liquid was made neutral to phenolphthalein with sodium hydroxide and mixed with 20 c.c. of a molar/15 phosphate solution having a P_H value of 7.7. A gram of powdered fibrin was added and shaken until soaked and swollen, after which the mixture was made to 50 c.c. and incubated through three hours at 40°. A parallel blank test was always made on an equal volume of the stomach contents, but boiled to kill the ferment present. After the incubation the 50 c.c. digested mixtures were chilled to stop action, poured into 100 c.c. cylinders containing sufficient volume of barium hydroxide and chloride solution to precipitate all the phosphate present, and made up to the mark with water. The cylinders were allowed to stand overnight to permit the precipitates to settle, after which 50 c.c. portions were poured or filtered off, neutralized to phenolphthalein and titrated with 0.2 *N* alkali in presence of formaldehyde in the usual manner. From the results of the titrations in the unboiled samples the blank results were subtracted. The differences multiplied by 2 gave the titration values for the original portions (25 c.c. or less), measured from the stomach contents. The values for the whole contents were, in turn, calculated from these. In Table A the data, as so outlined, are given.

These tests were carried out over an extended period on one dog and over shorter periods on two other dogs. Many of the results were so near alike that they might be considered as duplicates. A large number of these are omitted from the table. The first column gives the date, the dog number and the amount of meat or egg added to the basic diet. In the next columns we have the weight of trypsin powder ingested and the time the mixture was allowed to remain in the stomach. The volume of withdrawn contents follows, and this is all that could be readily brought up by the tube. The next columns show the amount of free and combined acid present, calculated for the total volume. In some cases these values are

but close approximations only because a sufficiently large amount for fuller tests could not be spared from what was needed for the trypsin tests. The presence or absence of bile is indicated in the next column and then the amount taken for each trypsin test, equal volumes being employed in the direct and boiled tests. In the following columns the alkali required in each titration is shown and then the net result for the volume measured out, $2\ (a-b)$. The difference between the two titrations is doubled because only half of the measured volume is actually used in each case. The last column gives the amino nitrogen liberated, calculated for the whole stomach content.

The results as a whole are interesting. When trypsin is not ingested the amount of nitrogen in amino combination liberated from the substrate fibrin is always minute, except when there is evidence from the appearance of bile that proteolytic ferment may have entered the stomach from the intestine. Even here the tryptic activity is not strong in any case. In one of the dogs the tendency to regurgitation was seldom noticed, while in another, No. I, it was rather common, as in man. It is safe to conclude from these experiments that when trypsin is not ingested the tryptic activity of the stomach is so slight as to be negligible for our purpose. The large number of experiments here included point clearly to this conclusion. It was thought desirable to secure the data over an extended period so as to be able to reach a fair degree of certainty and settle the question satisfactorily.

On the other hand when trypsin is ingested with the meal we have two conditions to consider. In the one case we have the test meal of cracker crumbs and water alone, and in the other the addition of meat in relatively large amount. When meat was not fed the accumulation of free acid in the stomach was usually marked, amounting in one instance to 0.42 per cent. With the meat present the acid was naturally found to be largely combined. In these we observed the highest tryptic activity in the aspirated liquid, and this in many cases was very marked. At first sight the

TABLE A

			Trypsin Gm.	Time in Stomach	Vol. of Liquid Drawn	Free HCl	Combined HCl	Bile Present	Volume Taken for Trypsin Test	C.c. N/5 NaOH Used for Original (*a*)	C.c. N/5 NaOH Used for Blank (*b*)	2 (*a*—*b*)	Indicated Gm. of NH_2N for Whole Stomach Content Activity
III.	April 25,	100 gm. meat	1	45	80	0.000	0.584	—	25	15.2	9.9	10.6	0.065
III.	April 26,	no meat	0	45	30	0.033	0.550	—	10	0.8	0.6	0.4	0.003[1]
III.	April 27,	100 gm. meat	1	60	80	0.000	0.443	—	25	11.3	3.3	16.0	0.145
III.	May 1,	60 gm. egg	1	60	60	0.000	0.219	—	20	4.6	2.1	5.0	0.042
III.	May 2,	100 gm. meat	0.5	60	130	0.000	0.057	—	25	1.7	1.0	1.4	0.020[2]
III.	May 3,	100 gm. meat	1	60	80	0.000	0.292	—	25	7.6	3.4	8.4	0.065
III.	May 4,	no meat	0	60	90	0.261	0.033	—	25	4.1	4.0	0.2	0.001
III.	May 8,	50 gm. meat	1	45	110	0.000	1.121	—	25	3.1	1.9	2.4	0.030
III.	May 9,	100 gm. meat	1	75	29	0.000	0.084	—	10	3.0	0.9	4.2	0.054
III.	May 15,	50 gm. meat	1	50	37	0.000	0.055	—	15	2.7	1.9	3.4	0.024
III.	May 17,	100 gm. meat	1	60	32	0.000	0.108	—	15	4.2	2.1	4.2	0.025
III.	June 4,	100 gm. meat	1	60	130	0.000	0.303	—	25	8.6	3.5	10.2	0.136
III.	June 8,	no meat	1	45	110	0.000	0.160	—	25	4.1	2.5	3.2	0.089[3]
III.	June 12,	100 gm. meat	1	60	95	0.000	0.206	—	20	11.6	4.2	14.8	0.197
III.	June 13,	50 gm. meat	1	60	109	0.000	0.341	—	25	14.1	5.8	16.6	0.203
III.	June 23,	no meat	1	45	190	0.111	0.138	—	25	5.9	2.8	6.2	0.132
III.	June 26,	no meat	1	60	80	0.057	0.029	—	25	8.4	3.3	10.2	0.091
III.	June 28,	no meat	1	60	43	0.006	0.015	—	20	5.1	3.1	4.0	0.024[4]
III.	June 29,	no meat	1	60	44	0.184	0.158	—	20	9.0	5.2	7.6	0.047
III.	July 10,	100 gm. meat	0	75	92	0.000	0.168	—	30	3.1	3.1	0.0	0.000
III.	July 11,	50 gm. meat	0	90	100	0.000	0.282	—	40	2.3	2.0	0.6	0.004
III.	July 12,	50 gm. meat	0	120	56	0.000	0.036	—	25	1.2	1.2	0.0	0.000[5]
III.	July 13,	50 gm. meat	0	75	48	0.000	0.052	+	20	2.6	2.0	1.2	0.007
III.	July 14,	50 gm. meat	0	60	73	0.000	0.105	—	30	2.0	2.0	0.0	0.000

III.	Oct. 18,	no meat	0	45	170	0.171	0.122	—	25	2.9	2.9	0.0	0.000
III.	Oct. 19,	no meat	0	45	102	0.064	0.149	+	20	6.7	3.2	7.0	0.098
III.	Oct. 20,	no meat	0	50	85	0.433	0.047	—	20	0.9	0.9	0.0	0.000
I.	Oct. 16,	no meat	0	45	100	0.250	0.086	—	20	2.7	2.7	0.0	0.000
I.	Oct. 17,	no meat	0	50	80	0.134	0.056	+	20	1.4	1.0	0.8	0.008
I.	Oct. 18,	no meat	0	45	125	0.069	0.069	+	20	2.9	2.5	0.8	0.014
I.	Oct. 19,	no meat	0	45	150	0.075	0.255	+	20	4.7	2.6	4.2	0.085
I.	Oct. 20,	no meat	0	50	150	0.765	0.105	—	20	1.2	1.1	0.2	0.005
II.	Oct. 16,	no meat	0	45	150	0.327	0.225	—	20	2.1	2.1	0.0	0.000
II.	Oct. 17,	no meat	0	50	160	0.080	0.138	—	20	1.0	0.9	0.2	0.004
II.	Oct. 18,	no meat	0	45	130	0.234	0.065	—	20	1.6	1.6	0.0	0.000
II.	Oct. 19,	no meat	0	45	110	0.088	0.154	—	20	1.6	1.6	0.0	0.000
II.	Oct. 20,	no meat	0	50	100	0.150	0.130	—	20	1.1	1.1	0.0	0.000
III.	Oct. 23,	50 gm. meat	0	50	50	0.082	0.116	—	20	1.7	1.6	0.2	0.001
III.	Oct. 24,	100 gm. meat	0	55	75	0.109	0.218	—	25	2.7	2.7	0.0	0.000
III.	Oct. 25,	100 gm. meat	0	50	60	0.063	0.235	+	25	4.5	4.0	1.0	0.007
III.	Oct. 26,	100 gm. meat	0	55	112	0.033	0.547	—	25	1.9	1.9	0.0	0.000
III.	Oct. 27,	50 gm. meat	0	55	108	0.086	0.353	—	25	2.0	2.0	0.0	0.000
I.	Oct. 23,	50 gm. meat	0	45	100	0.000	0.480	+	25	2.4	1.3	2.2	0.025
I.	Oct. 24,	100 gm. meat	0	55	59	0.119	0.177	—	25	2.3	2.2	0.2	0.001
I.	Oct. 25,	100 gm. meat	0	50	72	0.014	0.321	+	25	4.0	2.9	2.2	0.018
I.	Oct. 26,	100 gm. meat	0	45	126	0.000	0.726	—	25	1.9	1.9	0.0	0.000
I.	Oct. 27,	50 gm. meat	0	55	115	0.000	0.529	+	25	2.1	1.6	1.0	0.013
II.	Oct. 23,	50 gm. meat	0	45	90	0.164	0.369	+	25	3.5	2.5	2.0	0.020
II.	Oct. 24,	100 gm. meat	0	55	100	0.236	0.136	—	25	1.8	1.8	0.0	0.000
II.	Oct. 25,	100 gm. meat	0	50	100	0.137	0.309	—	25	2.5	2.5	0.0	0.000
II.	Oct. 26,	100 gm. meat	0	45	120	0.086	0.546	—	25	2.1	2.1	0.0	0.000
II.	Oct. 27,	50 gm. meat	0	50	95	0.181	0.167	—	25	2.1	2.1	0.0	0.000

1. Stomach was washed out before meal was given.
2. The dog drank more water after the test meal.
3. Note that with no meat given there is no free acid in the stomach contents.
4. Not much left in stomach except mucus-like liquid.
5. Time in stomach too long. Gave water and aspirated this as the remaining liquid was but little.

recorded values would appear to be too high to be possible in a number of instances. For example, in the case of Dog III, on April 27 we note the liberation of 0.143 gm. of amino nitrogen from a fibrin substrate, and this is in excess of what might be expected from the gram of fibrin. It must be kept in mind, however, that the action on the gram of fibrin is about one seventh of this, from the small volume of contents used. The results in the last column, it must be remembered, are calculated for the whole contents.

In the case of Dog III, June 23, we find a high tryptic activity when no meat was fed and when the free hydrochloric acid had reached 0.06 per cent., and also on June 29 when the free acidity had reached 0.42 per cent., but ordinarily there is marked tryptic activity only in the presence of the protecting protein which holds the acid. The strongest evidence of tryptic digestive power is shown on June 12 and June 13 with Dog III when there was no free acid, but plenty of combined acid in the contents still remaining in the stomach.

It will be noticed that the times given for the reaction in the stomach were, ordinarily, forty-five to sixty minutes. More could not well be allowed with dogs as the contents pass rather rapidly into the duodenum, and even in the intervals chosen by us there was undoubtedly a considerable loss of contents in many cases. Taking the experiments as a whole, however, there is evidence that a good part of the proteolytic power of the administered trypsin persisted after this prolonged contact with acid and pepsin.

EXPERIMENTS AFTER LIGATION OF PYLORUS

We next carried out a number of experiments on two dogs in which the pylorus had been completely ligated so as to make a closed pouch of the stomach. After the abdominal operation to reach the organ the animals had been kept long enough in good condition to restore them to normal health and activity before the ligature was made.

Dog I.—After washing out the stomach by means of the tube a gram of trypsin was administered in a

soft capsule and the flow of secretion stimulated by injection of gastrin. After three hours 20 c.c. of a light colored liquid were drawn off, representing nearly the whole contents. No free acid was recognized, but some was held combined by the abundance of mucus present.

Trypsin tests were made on portions of 8 c.c. by the methods already described.

In direct test used 6.1 c.c. of 0.2 *N* NaOH. In blank, 3.7 c.c. of 0.2 *N* NaOH. Digestion increase, 2.4 c.c.

The amino acid nitrogen liberated by the trypsin remaining in the 20 c.c. of liquid would therefore amount to 0.037 gm., indicating the persistence of some of the trypsin through three hours. Other experiments on this dog were lost on account of repeated vomiting.

Dog II.—After washing the stomach the dog received a gram of trypsin and a gastrin injection. The animal was kept quite at rest through three hours and vomiting avoided. At the end of the time 80 c.c. of contents were withdrawn by the tube. In aliquot parts free and combined acid were determined, giving for the whole volume 0.086 gm. of free HCl and 0.029 gm. of combined acid. Trypsin tests were made on 25 c.c. portions with 1 gm. of the fibrin, as before. By the formaldehyde titration we found:

In direct test used 6.5 c.c. of 0.2 *N* NaOH. In blank, 3.5 c.c. of 0.2 *N* NaOH. Digestion increase, 3.0 c.c.

These figures correspond to a liberation of 0.054 gm. of amino acid N for the whole volume of contents.

With the same dog twenty-four hours later, after washing the stomach, a new trial was made, feeding 1 gm. of trypsin and following with the gastrin. After waiting three hours a volume of 72 c.c. of contents was brought up by the tube. The free HCl found was 0.157 and the combined 0.052 gm. For 25 c.c. portions we found by the formaldehyde titration:

In direct test used 5.7 c.c. of 0.2 *N* NaOH. In blank, 3.1 c.c. of 0.2 *N* NaOH. Digestion increase, 2.6 c.c.

This points to a liberation of 0.042 gm. of amino N for the whole volume.

On the following day, that is, forty-eight hours from the beginning of the experiment, the dog was still in good condition for continued trials. The stomach was washed out and gastrin injected. A meal consisting of 10 gm. of cooked meat, an egg, some water and a gram of trypsin was given. The dog was kept quiet through three hours and then the stomach content consisting of 60 c.c. of acid liquid was withdrawn. The total free acid amounted to 0.273 gm. and the combined acid to 0.224 gm., or about 3.5 per cent. of the weight of dry protein ingested. A marked flow of acid seemed to be secured by the combined action of the gastrin and the food protein, and the excess of free acid in the liquid amounted to about 0.45 per cent. of the weight of the 60 c.c. collected. A test for trypsin was made here as before, but in this case the formaldehyde titration gave no evidence of the liberation of amino acid nitrogen. It was apparent that the ferment had been destroyed by the acid and pepsin secreted.

The results of these experiments are very interesting. Here no trypsin could work back from the duodenum, yet we find a good reaction in the case of Dog I and in two of the trials with Dog II. In these three instances the accumulation of free acid seemed to be too small to inhibit the tryptic activity. For Dog I no free acid was found, while for the other animal it was a trifle over 0.1 per cent. on the first day and about 0.2 per cent. on the second, calculated for the whole liquid volume. Many earlier observations from this laboratory go to show that trypsin withstands pretty well a degree of acidity not greater than this in the absence of much pepsin. On the third day, however, the acidity was markedly high, although not unusual in the dog, and here we find that the ferment was all destroyed, or so much weakened as to give no positive test. The conditions for the activation of pepsin by acid to the point where it will destroy trypsin, as shown by experiments in vitro, are indicated in our last paper.[1] Not much tryptic activity

should be expected in any of the above experiments. That any at all is found in presence of the acid and pepsin, indicated by the qualitative tests made, points to a rather marked degree of tryptic persistence.

EXPERIMENTS ON A DOG WITH A GASTRIC FISTULA

In this series we employed one dog throughout, Dog VI on our list. An operation was made in March on an animal in good condition. The wound healed perfectly and in the course of about three weeks the animal appeared perfectly normal, with good appetite. The pylorus was not touched and the flow remained unobstructed.

A diet shown in the table attached, Table B, was fed along with variable amounts of water. The animal was kept in a hammock after the feeding until the time of observation was completed. Then the liquid remaining in the stomach was drawn off for tests. By waiting too long we frequently found that practically everything had left the stomach. In some of these experiments, as in others, the flow of an increased amount of juice was stimulated by the injection of gastrin. In the tests a good pepsin reaction was always noted, but bile was generally absent. No column is kept for these tests in the table, but the few times when the bile was observed is stated in footnotes, as this may suggest regurgitated trypsin. The methods of testing employed were the same as in the previous cases. See Table B.

The evidence for the persistence of trypsin in the presence of pepsin and acid is as clear from these experiments as from the earlier ones. When trypsin was not administered it was not found in the stomach contents except in traces accompanying bile from the duodenum. In only a few instances of administration do we fail to find it in the stomach fluid, and then only when the time elapsed was long enough to allow the stomach material to go into the duodenum or when the acid secretion was pretty high. Such a case appears on June 26, when the time was long and the remaining contents small in volume but high in percentage acidity. On June 7 and 8 the acid content of

TABLE B

	Trypsin Given	Time in Stomach	Volume of Liquid Drawn	Free HCl	Combined HCl	Volume Taken for Trypsin Test	C.c. N/5 NaOH Used for Original (*a*)	C.c. N/5 NaOH Used for Blank (*b*)	(*a*−*b*)	Indicated Gm. of NH_2. N for Activity of Whole Content
May 2, no food	0	60	15	0.048	Trace	5	1.0	1.0	0.0	0.000[1]
May 2, meat and milk	1	60	50	0.055	0.091	5	2.3	1.1	2.4	0.067
May 3, 80 gm. meat	1	60	25	0.000	0.081	5	2.9	1.5	2.8	0.039
May 4, 50 gm. meat, 1 egg	0	120	80	0.000	0.451	25	3.7	3.7	0.0	0.000
May 8, 100 gm. raw meat	0	90	12	0.000	0.018	3	0.6	0.3	0.6	0.007[2]
May 9, water only	0	45	13	0.000	0.002	5	0.6	0.4	0.4	0.002[3]
May 15, 20 gm. meat	0	50	8	0.000	0.017	3	2.1	2.1	0.0	0.000
May 16, 50 gm. meat, 2 eggs	1	60	172	0.000	0.940	25	4.2	3.1	2.2	0.042
May 17, 50 gm. meat, 1 egg	1	90	150	0.000	0.573	25	5.0	4.1	1.8	0.030
May 24, 50 gm. meat, 1 egg	1	60	90	0.049	0.115	25	5.6	3.6	4.0	0.040
June 1, no food, gastrin	0	90	16	0.043	Trace	5	0.2	0.2	0.0	0.000
June 4, no food, gastrin	0	120	37	0.215	Trace	5	0.2	0.2	0.0	0.000
June 7, no food, gastrin	1	30	14	0.079	0.000	6	1.5	1.0	1.0	0.007
June 8, no food, gastrin	1	30	9	0.048	0.000	3	3.1	1.9	2.4	0.026[4]
June 12, 50 gm. meat, 1 egg	1	60	105	0.063	0.471	25	6.6	5.0	3.2	0.039
June 13, 50 gm. meat, 2 eggs	1	120	75	0.066	0.200	25	9.8	5.0	9.6	0.081[5]
June 15, 50 gm. meat, 2 eggs	1	120	61	0.178	0.266	25	8.1	5.2	5.8	0.040[6]
June 19, 50 gm. meat, 2 eggs	1	120	83	0.046	0.175	25	7.4	4.9	5.0	0.047
June 21, 50 gm. meat, 1 egg	1	75	52	0.058	0.116	20	8.0	3.9	8.2	0.080
June 23, 2 eggs, much water	1	60	260	0.000	0.286	25	5.2	2.5	5.4	0.157
June 26, 50 gm. meat	1	105	16	0.035	0.018	7	2.1	2.1	0.0	0.000
June 28, 50 gm. meat	1	60	75	0.164	0.410	25	16.7	7.6	18.2	0.163
June 29, 1 egg	1	60	100	0.000	0.218	20	4.6	2.0	5.2	0.078
July 11, 50 gm. meat, 2 eggs	0	120	75	0.027	0.216	30	4.8	4.8	0.0	0.000
July 12, 50 gm. meat, 1 egg	0	120	56	0.010	0.101	20	4.6	4.6	0.0	0.000
July 13, 50 gm. meat, 1 egg	0	60	67	0.034	0.194	25	5.0	3.0	6.0	0.090

1. Initial juice after washing out the stomach.
2. A little bile was present and possibly trypsin from regurgitation.
3. Bile present. The slight tryptic action may be due to duodenal contents.
4. Note persistence of some tryptic action in presence of 0.5 per cent. HCl.
5. Note tryptic action after 2 hours.
6. Same point in this and the following experiment.

the liquid brought up was about 0.5 per cent. Such a concentration in presence of pepsin is usually speedily destructive, and there was no protecting protein here. The highest residual tryptic activity is noted on June 23 and June 28, when the time was an hour. Free acid was absent in one case, but was rather high in the other.

It will be noted that the figures for liberated amino acid nitrogen appear rather low in many instances. In general, however, they represent a good degree of tryptic activity, because in every case a large part of the stomach contents and added trypsin had undoubtedly passed unhindered into the duodenum. The liquid volumes secured for the tests simply represent what had not escaped in this manner.

REACTIONS IN A PAWLOW POUCH

Finally we took up the problem of the behavior of trypsin in contact with acid and pepsin from another angle. To avoid entirely the complication from the possible presence of duodenal trypsin in the stomach, as a consequence of regurgitation, we have made use of a false stomach or Pawlow pouch in a dog.[3] Our first plan of procedure was to place in this pouch small amounts of liquid and protein plus the trypsin for observation. A good secretion of acid and pepsin was always secured in the pouch and the action of this on the trypsin and protein was observed after a time. But the plan was not satisfactory for observations of any length as the secreted liquid could not be retained in the pouch except when the dog was kept on its side or in some other uncomfortable position Finally this scheme was adopted: The secreted juice was allowed to flow as fast as formed from the false stomach into a small bottle held in a container strapped to the body of the animal. At the outset the trypsin, when used, and a constant weight of cooked and finely ground meat, 5 gm., were placed in the bottle which was held in the proper position through three hours,

3. Our thanks are due to Dr. W. Keeton of the Department of Pharmacology for the work of this operation and for much other valuable help during the progress of the investigation.

the dog being supported in a hammock meanwhile. In this manner the bottle constituted a third stomach which was maintained at the body temperature by the circulation of water in the container outside. Besides being bound tightly to the body of the animal, the metallic jacket was surrounded by pads to assist in retaining the right warmth.

Under these conditions a considerable flow of a normal gastric juice took place into the glass stomach, which had to some extent the motions of the dog's abdomen. At the beginning of the experiment the animal was fed in the usual way and generally received 200 gm. of meat and an egg. The larger flow of the gastric juice naturally went to the normal stomach, but there was always an appreciable amount in the other direction to be collected finally in the bottle. Table C gives the results of the tests made on the bottle contents. The 5 gm. of meat were usually well digested, pointing to the activity of the collected juice. The tests for tryptic action in the resulting liquid were made as before on a substrate of 1 gm. of fibrin in the phosphate mixture.

The figures here reported seem to show a rather high degree of proteolytic activity in the trypsin remaining in the bottle after having been subjected to the influence of the pepsin and the hydrochloric acid. The substrate for the final test was the gram of fibrin, and the amino acid N reported is more than could be furnished by that weight of protein. But the same explanation holds here as before. The values in the last column show the nitrogen which would be liberated from the whole of the liquid collected in the bottle, while the gram of fibrin is acted upon by a fraction only of this volume. It must be recognized, in addition, that the substrate is in reality more than the fibrin since the meat in the glass stomach has been largely liquefied in the operation and must be attacked also by the trypsin. In some cases as much as 2 gm. of meat go in this way to form part of the substrate.

In a few cases the combined hydrochloric acid calculated for the whole flow appears very high. The collecting bottle contains the protein of the trypsin as

well as the meat, but the latter has generally undergone partial digestion, leaving substances which have a higher binding power than the original protein. It is also to be noted that the acid secreted was often in a state of partial combination with mucus or some other protein-holding substance. This is illustrated in the collection of July 11, for example, where neither meat nor trypsin was put in the bottle. In any event the secretion of acid in this dog must be considered as relatively large.

TABLE C

	Tryp- Jar	Juice C.c.	HCl	Com- bined HCl	Volume for Tryp- sin Test	C.c. N/5 NaOH for Original (*a*)	C.c. N/5 NaOH for Blank (*b*)	2 (*a*—*b*)	Indicated Gm. of NH_2, N for Activity of Whole Collection
April 27	1.0	50	0.000	0.232	20	21.5	6.0	31.0	0.217
May 1	1.0	25	0.000	0.139	10	16.6	5.6	22.0	0.154
May 2	0.5	31	0.000	0.112	10	13.7	4.5	18.4	0.160
May 3	0.5	22	0.000	0.120	10	13.5	4.5	18.0	0.111
May 4	0.0	25	0.105	0.011	10	2.3	2.3	0.0	0.000[1]
May 8	0.5	27	0.027	0.377	10	2.8	2.6	0.4	0.004[2]
May 9	0.5	34	0.000	0.172	10	3.2	3.0	0.4	0.003[2]
May 15	0.5	42	0.061	0.305	10	2.6	2.5	0.2	0.002[2]
May 16	1.0	58	0.042	0.190	10	4.1	4.0	0.2	0.003[2]
May 22	1.0	56	0.000	0.184	25	4.0	4.0	0.0	0.000[2]
May 24	1.0	55	0.040	0.460	25	15.4	7.4	16.0	0.099
May 31	1.0	41	0.000	0.447	15	13.0	5.6	14.8	0.113
June 1	1.0	40	0.000	0.364	15	14.1	7.4	13.4	0.100
June 4	1.0	45	0.006	0.426	20	14.2	5.6	17.2	0.108
June 6	1.0	50	0.027	0.473	20	15.9	5.9	20.0	0.140
June 7	1.0	46	0.000	0.218	20	12.3	5.9	12.8	0.082
June 8	1.0	50	0.021	0.291	20	13.0	6.0	14.0	0.098
June 12	1.0	44	0.016	0.321	20	17.0	4.4	25.2	0.165
June 13	1.0	50	0.013	0.418	20	13.9	6.0	15.8	0.110
June 15	1.0	53	0.058	0.270	20	14.9	7.1	15.6	0.116
June 19	1.0	35	0.013	0.078	15	14.2	5.4	17.6	0.115
June 21	1.0	45	0.031	0.106	20	18.2	6.9	22.6	0.142
June 23	1.0	51	0.074	0.158	20	10.3	1.9	16.8	0.120
June 26	1.0	45	0.033	0.131	20	12.3	7.6	9.4	0.059
June 27	1.0	57	0.031	0.207	25	11.4	5.3	12.2	0.077
June 28	1.0	50	0.018	0.180	20	11.4	4.6	13.6	0.095
July 11	0.0	41	0.056	0.128	15	3.0	3.0	0.0	0.000[3]
July 12	0.0	50	0.273	0.018	20	1.9	1.9	0.0	0.000[3]
July 13	0.0	45	0.147	0.066	15	1.3	1.3	0.0	0.000[3]

1. Juice collected through 1 hour only.

2. In all these cases the trypsin was killed by heat before being added to the bottle. Note the high combined acid in the collection of May 8 and on several of the following dates.

3. In these cases no meat was placed in the collecting bottle, as well as no trypsin. The low results of the titrations probably point to the presence of protein in the secreted juice, as is suggested by the high combined acid noted in some cases.

Giving due weight to all the factors in this case it is still evident that the trypsin has exhibited a marked resisting power to the combined action of the acid and pepsin in the presence of protein. The large number of tests recorded show that the results are not accidental. While rather marked differences in the extent of the fibrin digestion are recognized, there is always some action and usually decided where unkilled trypsin was used. In addition to this there is some evidence suggesting that the trypsin may even aid in the digestion and liquefaction of the meat placed in the bottle. This is the case where the free acid is rather low, below the point where it is capable of inhibiting the proteolytic action of the pancreas enzyme. It is not likely that pepsin and trypsin can act simultaneously as the hydrogen-ion concentration which makes peptic digestion possible, is above the level where tryptic action is appreciable or possible. But with a denatured protein like cooked meat, trypsin may act before the hydrochloric acid has accumulated to the inhibiting point, or after it has become largely combined with the split products.

In the formaldehyde titrations the so-called blanks are high in most cases. Here we have the groups originally present or which are formed by the peptic digestion in the preliminary stage. The tests of May 4, 8, 9, 15, 16 and 22 disclose low blanks because either no trypsin or inactive trypsin was present. On the other dates the much higher blanks suggest that we must have some little splitting of amino bonds even in the preliminary or acid stage. These blanks are approximately twice as high as in the no-trypsin cases and are most satisfactorily explained on this hypothesis. The collections of April 27, May 24, June 1, June 15, June 21 and June 26 are good illustrations. When the other tables are analyzed some similar cases are found.

CONCLUSIONS

In these experiments carried out on dogs to determine the combined effect of hydrochloric acid and pepsin on trypsin, under conditions which correspond to those obtained in the human stomach at times, when the latter ferment is ingested, four lines of observations were followed.

In these four groups of observations, after the ingestion of trypsin, the stomach contents were secured (*a*) by means of a tube after the ligation of the pylorus, in which case the organ constituted a closed pouch in which the secretion followed normally for a time, (*b*) from the normal open stomach by the tube applied at the proper interval after the ingestion of food and added trypsin, (*c*) by means of a gastric fistula made in the normal organ and opened from time to time for the withdrawal of contents, and (*d*) from a false stomach or Pawlow pouch constructed from the normal organ.

In all the animals the secretion of pepsin and acid was abundant, and from this point of view the conditions for the persistence of trypsin were not favorable. Yet, in the larger number of experiments, this latter ferment was not destroyed by the other combination where sufficient protein was present to bring the concentration of the free acid down to a certain value. Trypsin seemed to be destroyed or greatly weakened only when the acid was in excess with pepsin.

These experiments appear to confirm our earlier conclusions from work done in vitro that trypsin, pepsin and hydrochloric acid may exist side by side under conditions which, following the ingestion of trypsin, may exist in the human stomach. It is even possible that some trypsin proteolysis may occur then in that organ when the free acid is very low from protein combination. The destruction or weakening of the trypsin is a function, probably, of the hydrogen-ion concentration.

A CONTRIBUTION TO THE PHARMACOLOGY OF STOVAINE*

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AND

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(*From the Journal of Pharmacology and Experimental Therapeutics, January, 1917*)

There is an extensive literature relating to the uses of stovaine as a local anesthetic, and more especially in spinal anesthesia, but its behavior in the body after its absorption into the blood stream has received little consideration at the hands of pharmacologists, and we are in need of more definite knowledge of its relative toxicity and anesthetic activity as compared with cocain and other locally acting members of this series.

The following brief references to the literature will serve to show the need of further information along the lines just mentioned.

Münchmeyer[1] states, as his conclusions from the study of 1,189 cases of spinal anesthesia with stovaine, that not more than 70 per cent. are entirely free from by, or after, effects. One patient died from respiratory failure and seven suffered transient paralysis.

Madden[2] mentions three fatalities following the use of stovaine in spinal anesthesia in doses of 40 to 60 mg., failure of respiration being the immediate cause of death.

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* This is the second of a series of papers dealing with the pharmacology of the local anesthetics, the first one, on novocain by Hatcher and Eggleston, appeared in the Journal of Pharmacolgy and Experimental Therapeutics, 1916, **8**, 385.

* Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Münchmeyer, O.: Beitr. z. klin. Chir., 1908, **5**, lix.
2. Madden, F. C.: Brit. Med. Jour., 1912, **2**, 345.

Hardouin[3] mentions sixteen fatalities attending the use of stovaine, and he believes that the drug is certainly responsible for at least eight of these deaths which occurred after doses varying from 40 to 80 mg.

We might mention other accidents that have followed the use of ordinary doses of stovaine, but, on the other hand, various authors have reported the survival of much larger amounts, thus Sabadini[4] records a case in which a dose of 150 mg. was given by mistake. This was followed by dyspnea, cyanosis, a thready pulse, pallor and faintness, with complete recovery.

Veley and Symes[5] studied the toxicity of stovaine in animals and found that small but effective doses produce a fall in blood pressure and a pause in the respiration. As little as 5 mg. per kilogram proved fatal to anesthetized cats by intravenous administration, but as much as 40 mg. per kilogram might be survived if given in fractional doses. Baylac[6] also observed the greater tolerance toward fractional doses and suggested that the drug is destroyed or modified in the organism.

Pouchet and Chevalier[7] state, that stovaine lowers the blood pressure and causes acceleration of the heart, but they conclude that it exerts a tonic action on the heart. Kamenzove[8] observed that stovaine causes a fall of blood pressure in dogs and rabbits and attributes this to a vasodilator action of the drug, inasmuch as Pouchet and Chevalier had reported that it did not exert a toxic action on the heart directly.

THE ACTION OF STOVAINE ON THE HEART AND CIRCULATION

The symptoms following the intravenous administration of a toxic dose of stovaine point so strongly toward an injurious action directly on the heart that this subject merits a brief discussion here.

3. Hardouin, P.: Arch. gén. de chir., 1908, **2**, 115.
4. Sabadini: Lancet, 1908, **2**, 1215.
5. Veley, V. H., and Symes, W. L.: Proc. Roy. Med. and Chir. Soc., 1910, s. B. **83**, 413.
6. Baylac, M. J.: Compte rend. Soc. de biol., 1906, **60**, 254.
7. Pouchet and Chevalier: Bull. gén. de thérap., 1904, **148**, 36.
8. Kamenzove, Z.: Arch. int. de pharm. et de thérap., 1911, **21**, 5.

Intravenous injections of doses of 5, 10, and 20 mg. of stovaine per kilogram respectively caused declines of 30, 55, and 70 per cent. in the blood pressure of a cat that had been anesthetized with ether. The heart beats became weaker after the smallest of these doses; the second caused irregularities in the rate and force; the third was followed at once by slight irregularities in the rate, the beats becoming weaker and gradual failure resulting. It is hardly necessary to say that the effects of the preceding dose had passed when the next was given. The injection of a dose of 10 mg. per kilogram during anesthesia in another experiment caused the blood pressure to fall rapidly, with a brief return of the pressure to its previous level and complete failure of the heart within one minute following the injection of the drug.

The cardiac effects of larger intravenous doses of stovaine in the normal animal are much like those just described. The immediate and simultaneous failure of the heart and respiration after the intravenous injection of large doses shows that the drug acts directly on the heart and on the respiratory center. This view is supported by the results of an experiment in which the rabbit's heart was perfused with a modified Locke's solution,[9] to which had been added 1 part of stovaine to 10,000 of the perfusing fluid. The effects on the heart are shown in Figure 1. The heart rate was slowed slightly, the amplitude of contraction was greatly diminished.

The chief cardiac effects are independent of any action on the vagus, since they occur even when atropin has been given previously in doses sufficient to paralyze the vagus endings.

Stovaine is commonly said to cause dilation of the vessels at the point of injection when it is used subcutaneously in man, but this action does not appear to be exerted directly on the vessels when the drug

9. The formula of the solution used in this experiment was as follows: sodium chlorid, 0.9 per cent.; potassium chlorid, 0.042 per cent.; calcium chlorid, 0.024 per cent.; sodium bicarbonate, 0.05 per cent. That injected during exsanguination of the cats mentioned later was essentially similar except that it contained only 0.03 per cent. of sodium bicarbonate, and in addition, 0.1 per cent. of glucose.

enters the blood stream, in which case the concentration is far less than that in which it acts locally after the subcutaneous injection in clinical practice.

Several experiments were conducted in which the outflow from the renal veins was measured during perfusion with normal salt solution before and after the addition of stovaine in concentrations varying from 1 : 10,000 to 1 : 2,500. Not the slightest influence could be detected in these experiments, hence it is fair to assume that any direct dilator action on the vessels that stovaine may exert is too slight to be of impor-

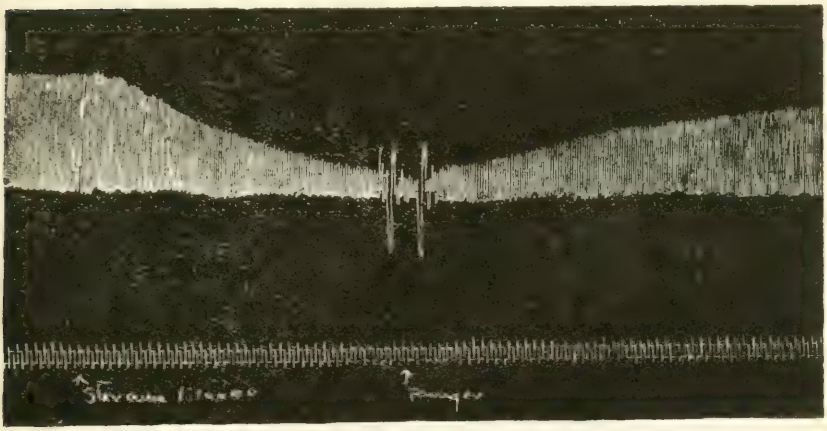

Fig. 1.—Perfusion of the rabbit's heart with one part of stovaine in 10,000 parts of Locke's solution. Prompt recovery begins when unpoisoned Locke's solution is substituted.

tance in the fall of blood pressure that results from the intravenous injection of toxic doses, and we must assume that the effect is almost wholly of cardiac origin in such cases.

TOXICITY OF STOVAINE BY VARIOUS MODES OF ADMINISTRATION

The toxicity of stovaine was studied on cats, rabbits, and guinea-pigs, the drug being administered subcutaneously and intravenously, in solutions of different concentrations.

The drug is absorbed rapidly from the subcutaneous tissues of the cat, and clonic convulsions resulted in two minutes, and death in six minutes, after the subcutaneous injection of a dose equal to 500 mg. of stovaine per kilogram of weight, dissolved in 10 parts of normal salt solution. The subcutaneous injection of a dose of 150 mg. per kilogram caused emesis and dyspnea in five minutes in the cat, this being followed by rapid recovery. A dose of 300 mg. per kilogram administered subcutaneously to another cat caused dyspnea and restlessness in five minutes, with labored respiration and severe clonic convulsions in twelve minutes and muscular weakness persisting for more than an hour. This was followed by complete recovery.

It is well known that the addition of epinephrin to a solution of stovaine delays its absorption, and there is no reason to doubt that even larger doses of stovaine than those mentioned here might be administered subcutaneously in combination with epinephrin to cats without causing death, but it did not seem worth while to extend the experiments in that direction.

The toxicity of stovaine by intravenous injection was determined by injecting solutions of different concentrations into the femoral veins of cats, rabbits, and guinea-pigs.

There was little difference observed in the activity of the drug when solutions of 1 : 10 and 1 : 100 were used, since the injection may be made practically instantaneously in either case, but with the longer time required for the injection of such dilute solutions as 1 : 500 there is a notable diminution of activity. The protocols in brief of two experiments will serve to show the rapidity of the action and the degree of activity of the drug under these conditions.

Male cat, weight 2 kg.

2 : 40 p. m. 30 mg. stovaine × kg. by vein in 1 : 10 normal salt solution.

2 : 40½ p. m. Heart beat imperceptible to the finger; the respiration continued for a few seconds longer.

Female cat, weight 3.4 kg.

11:32 a. m. Began injection of stovaine by vein in 1:500 normal salt solution.

11:35 a. m. Completed injection of dose of 35 mg. per kilogram. Clonic convulsions, rapid respiration and feeble heart beat, followed by complete recovery.

The injection of a dose of 30 mg. per kilogram into the ear vein of a rabbit in 50 parts of salt solution was almost immediately fatal. Guinea-pigs succumb promptly to doses of 40 mg. of stovaine per kilogram of weight when 1 per cent. solutions are injected intravenously.

The great difference observed in the amounts of stovaine required by subcutaneous and intravenous injection to cause death, despite the fact that absorption follows the subcutaneous injection promptly, suggested that stovaine may behave like novocain when fractional doses are administered intravenously, and this was found to be the case in the main, though complete recovery does not appear to follow the injection of such fractional doses of stovaine so promptly as in the case of novocain, as reported by Hatcher and Eggleston.

The protocol in brief of an experiment will serve to illustrate this point.

Male cat, weight 3 kg.

1:52 p. m. 10 mg. stovaine per kilogram 1:100 normal salt solution.

2:00 p. m. 10 mg. stovaine per kilogram as before.

2:08 p. m. 10 mg. stovaine per kilogram as before.

2:15 p. m. 10 mg. stovaine per kilogram as before.

2:23 p. m. 20 mg. stovaine per kilogram as before. Repeated clonic convulsions after thirty seconds and lasting about seven minutes.

2:35 p. m. 25 mg. stovaine per kilogram as before. Convulsions and death in about thirty seconds.

Similar results were observed when fractional doses of stovaine were administered to the rabbit by the ear vein, in which case complete recovery followed the administration of an amount equal to 72 mg. per kilogram within a period of about two hours.

DISAPPEARANCE OF STOVAINE FROM THE BLOOD STREAM

The facts already recorded point strongly to the rapid disappearance of stovaine from the circulating blood, and we undertook to confirm this by direct experiment.

A cat weighing 2.7 kg. received a total of 430 mg. of stovaine intravenously within a period of about three and a half hours; thirty minutes later it was exsanguinated and the blood defibrinated.

Fifty c.c. of the defibrinated blood (approximately one fourth of the blood mass of the animal) were added to 450 c.c. of alcohol, the mixture filtered, the filtrate evaporated and the residue dissolved in normal salt solution and tested biologically, advantage being taken of the fact that the cat reacts typically and fairly uniformly to stovaine when it is injected intravenously.

The intravenous injection of the whole of this solution of the residue failed to produce any symptoms in a cat weighing 0.7 kg. It was estimated that the solution thus prepared from the defibrinated blood contained about one fifth of the total amount of stovaine that remained in the circulation at the time of exsanguination, but since as little as 15 mg. would have induced symptoms in the cat used for the test we must suppose that less than 75 mg. remained in the blood stream after the injection of nearly six times that amount; as a matter of fact, we have reason to suppose that it is removed even more rapidly than the experiment just mentioned would seem to indicate.

In this connection it is hardly necessary to state that a control experiment was made in which stovaine was added directly to blood, and recovered almost quantitatively as shown by the method just described.

ELIMINATION OF STOVAINE BY THE KIDNEY

While it seemed highly improbable to us that the kidneys could eliminate stovaine as rapidly as it appeared to leave the blood stream, it was a simple matter to test the urine of the cat which received the

430 mg. of stovaine and which was exsanguinated for the test just described. The urine of this cat was collected during the period of the experiment, and all of it (including that remaining in the bladder at death) was injected into the femoral vein of the same cat that had been previously used for testing the presence of the stovaine in the blood. The result was entirely negative and we may say with certainty that not more than a very small proportion if any of the stovaine was excreted unchanged by the kidneys.

DESTRUCTION OF STOVAINE IN THE LIVER

The method used for determining whether stovaine is destroyed in the liver is practically like that employed by Hatcher and Eggleston in the investigation of the same problem with reference to novocain.

Three of these experiments were carried out with stovaine, the results being so nearly similar that only one of the experiments need be detailed.

Two cats weighing 3.46 and 1.9 kg., respectively, were exsanguinated while normal salt solution was being injected into the femoral vein. The blood thus obtained measured 250 c.c. after defibrination. Of this 200 c.c. to which had been added 600 mg. of stovaine, were used for the perfusion of the liver of the smaller cat which had been washed nearly free of blood by passing normal salt solution through it. The perfusion was maintained for two hours at a uniform temperature of 39 C., during which time the fluid passed through the liver about fifteen times, being shaken with oxygen until bright red after each passage through the liver.

The fluid obtained after the perfusion measured 185 c.c. A portion of this was used in the following experiment to determine whether any considerable part of the stovaine had been removed during the perfusion.

Cat, weight 1.7 kg.

2:24 to 2:27 p. m. Injected into femoral vein 30 c.c. of fluid per kilogram.

2:27 p. m. Injected into femoral vein 15 c.c. of fluid per kilogram. No symptoms could be perceived.

Each cubic centimeter of the fluid contained 3 mg. of stovaine before the perfusion, hence there would have been 135 mg. in the 45 c.c. of fluid injected for each kilogram of weight of the test animal had none been fixed or destroyed by the liver, and since as little as 20 mg. per kilogram suffices to induce symptoms, it follows that at least five sixths of the stovaine had left the perfused fluid.

We were unable to extract any of the stovaine from the liver and demonstrate its presence in the extract in one of these experiments, though a control experiment, in which stovaine was mixed with liver tissue and extracted, served to show that the drug could be recovered and its amount estimated fairly accurately by means of the biologic test.

The perfused fluid was tested directly on animals in two of the experiments, and in two extracts were prepared, and these were used for the biological tests. The method of extraction has been described in connection with the observations on the rate of elimination of the drug from the blood stream. In two of the perfusion experiments Locke's solution was injected into the cats during the exsanguination for obtaining fluid for perfusion, and in each of these two experiments only one cat was exsanguinated.

From the results of these perfusion experiments we conclude that stovaine is either destroyed or fixed in the liver, the weight of evidence being in favor of the view that it is destroyed in that organ.

SUMMARY

1. Our experiments afford no evidence that stovaine exerts any direct action on the blood vessels after the intravenous injection in cats, and it failed to change the caliber of the renal vessels of the cat or dog when perfused in concentrations of 1:10,000 to 1:2,500. It depresses the heart when toxic doses are injected intravenously, and when the rabbit's heart is perfused with a solution containing 1 part of the drug in 10,000 parts of Locke's solution.

2. Stovaine causes death by inducing immediate and simultaneous paralysis of the heart and respiration, the action on each being independent of that on the other.

3. Stovaine disappears rapidly from the blood stream after its intravenous injection.

4. Little, or none, of the drug is excreted unchanged in the urine of the cat.

5. Stovaine is removed from perfused fluid by the liver in which it appears to be destroyed.

6. The fatal dose of stovaine for the cat or rabbit is about 30 mg. per kilogram when a solution of 1 : 100 is injected rapidly into a vein. Somewhat more is required when dilute solutions are used. Complete recovery follows the injection of a toxic, but not fatal, dose within a short time, and several times as much as a single fatal dose may be administered within a few hours if small portions be given at short intervals. Very large doses are required by subcutaneous injection to cause death.

7. Stovaine is slightly, but distinctly, more toxic than novocain by similar modes of administration, and complete recovery does not follow the administration of toxic doses of stovaine so promptly as it does that of corresponding doses of novocain.

THE SALICYLATES

IV. SALICYLATE IN THE BLOOD AND JOINT FLUID OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF THE DRUG

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(*From the Journal of Pharmacology and Experimental Therapeutics, January, 1917*)

It has been suggested that the beneficial action of salicylates in rheumatic fever is connected with a difference in the distribution of the salicyl between the blood and joint fluid, namely, that there is a higher concentration of salicyl in the effusions of infected joints. Bondi and Jacoby[1] think they have shown this to be true in rabbits. They studied the distribution of salicylate in normal and infected rabbits, and found that the joints of rabbits infected with staphylococci contained more salicylate than those of healthy animals. This evidence cannot be considered as entirely conclusive, since the musculature, and particularly the blood and serum, contained the highest proportion of the salicylate, and this would have to be considered in connection with infected joints with serous effusions.

This proposition was put to a test by estimating the concentration of salicylate in the blood and joint fluid of individuals suffering with rheumatic fever and receiving full therapeutic doses of sodium salicylate. For this the distillation-colorimetric method was used.[2] By this method it is possible to recover 90 to 95 per cent. of such small quantities as 2 to 5 mg. of sali-

1. Bondi and Jacoby: Beitr. z. chem. Phys. u. Path., 1906, **7**, 514.
2. Thoburn and Hanzlik: Jour. Biol. Chem., 1915, **23**, 163.

cylate added to 10 to 20 c.c. of whole blood. The fluids were secured at the time of "toxicity" (deafness, ringing in the ears and nausea, etc.), that is, about eight hours after administration of the drug. Joint fluid was obtained by direct aspiration from the knee joints under aseptic precautions. Unfortunately our material (since October, 1914) has yielded only a small number of cases from whom sufficient fluid could be obtained to insure a reasonable degree of accuracy in the quantitative estimation. The joint fluids themselves (even though only a few drops were obtained) usually gave a positive test for salicylate with ferric alum. Blood was obtained from a vein in the arm. First, the results on the distribution of salicyl between the blood and joint fluid will be presented. These are summarized in Table 1.

TABLE 1.—DISTRIBUTION OF SALICYL* BETWEEN BLOOD AND JOINT FLUID

Patient and No.	Diagnosis	Total Quantity Salicyl Administered, Gm.	Blood			Joint Fluid		
			Quantity Used, C.c.	Total Quantity Salicyl Recovered, Gm.	Concentration of Salicyl, per Cent.	Quantity Used, C.c.	Total Quantity Salicyl Recovered, Gm.	Concentration of Salicyl, per Cent.
L2 (M. E.)	R. F.†	7.83 31.3 gm.‡ 2 days before	30	0.006	0.020	20	0.0035	0.018
L2 (L. E.)	R. F.	12.29	13	0.0018	0.014	13	0.0014	0.011
L1 (J. D.)	R. F.	10.06	10	0.0016	0.016	7	0.0008	0.011
8a (P. M.)	R. F.	14.00	10	0.0023	0.023	10	0.0025	0.025
8b (M. M.)	R. F.	4.5	20	0.006	0.030	15	0.0035	0.023
Average					0.020			0.018

* Expressed as salicylic acid.
† R. F. = rheumatic fever.
‡ This patient received 31.3 grams salicyl 2 days before the dose of 7.83 grams was administered.

The results indicate that the concentration of salicylate in both fluids does not differ markedly, being somewhat less, on the whole, for the joint fluid as might be expected. The following average concentrations were found; 0.02 per cent. for blood and 0.018 per cent. for joint fluid.

THE CONCENTRATION OF SALICYLATE IN THE BLOOD OF RHEUMATIC AND NONRHEUMATIC INDIVIDUALS

The method employed for estimating this was essentially the same as that described in the forepart of the paper. The results are presented in Table 2.

These seem to indicate a diminution in the concentration of salicylate in the blood of rheumatic as compared with nonrheumatic and normal individuals. The concentration in nonrheumatic and normal individuals ranged from 0.018 to 0.035 per cent., average 0.0265 per cent., and in rheumatics from 0.014 to 0.031 per cent., average 0.021 per cent. The average quantity of salicylate administered to both rheumatic and nonrheumatic individuals was about the same, i. e., about 13 grams (expressed as salicylic acid), and dividing this by the average weight of an adult, that is about 65 to 70 kg., would give a concentration of approximately 0.020 to 0.019 per cent., which compares favorably with what was found in rheumatic individuals, but not with nonrheumatic individuals in whom the concentration appears to be higher, namely, 0.026 per cent. Unfortunately, the weights of the majority of individuals were not secured. However, this was done in several instances. These together with other information are presented in the following tabulation.

Number and Patient	Body Weight Kg.	Total Salicyl* Administered, Gm.	Concentration Salicyl in Blood, Calculated, per Cent.	Concentration Salicyl in Blood, Found per Cent.
11 (S. V.)	47.8	17.0	0.0355	0.031
16b (M. C.)	53.6	11.4	0.021	0.018
16c (M. C.)	53.0	10.5	0.020	0.0186
20 (G. W.)	72.7	10.8	0.016	0.010
12 (W. B.)	46.4	8.75	0.0189	0.025
19 (Y. V.)	50.0	10.8	0.0216	0.024
21 (J. V.)	68.2	10.8	0.016	0.022
22 (E. L.)	62.7	10.8	0.0172	0.024

* In this tabulation, as elsewhere, "salicyl" means salicylic acid.

This shows that the concentration of salicyl is actually higher in some individuals than would be theoretically calculated from the body weight, assuming that the drug is evenly distributed throughout the body. For instance, Patients 12, 19, 21 and 22 showed a variable though definite increase in the concentration of salicyl in the blood. The possibilities to be considered in this are (1) unequal distribution of the salicyl; (2) an increase in the concentration of the blood at the time it was obtained for analysis, for in later experiments there has been demonstrated an increase in the percentage of hemoglobin of the blood of individuals receiving salicylate, and exhibiting marked diaphoresis about the time of "toxicity."

When the concentration is less, as in Patients 11, 16 and 20, for instance, three possibilities should be considered, (1) incomplete absorption, particularly of the last dose; (2) method of recovery, which entails a loss of 5 to 10 per cent; (3) destruction of the salicyl. It is not believed that incomplete absorption and the method will explain the diminution in concentration entirely. In our experiments the blood was always obtained some time (about one hour and even longer) after the last dose was administered, that is, only after the symptoms of "toxicity" were pronounced. Furthermore, salicylate is very rapidly and completely absorbed from the intestine as indicated by the exceedingly small and almost negligible recoveries (0 to 70 mg.), from the feces in a number (six) of individuals. Also the vomitus, when obtained (vomiting occurred only rarely — in two out of thirty patients), contained only a small quantity, up to 0.1 gram. These losses would not be sufficient to explain the diminution in concentration. So far as the method is concerned this was the same for all individuals and the percentage concentrations in the blood of a given group of patients approach a similar level with large or small quantities of blood. Finally, it is conceivable that salicyl is destroyed in the body. This would tend to reduce the concentration in the blood. In support of this we hope to present, in the near future, some data on the excretion of salicylate.

Taking the various possibilities into consideration it is not permissible, at this time, to formulate definite conclusions, but it is believed that, on the whole, the data indicate a tendency for the concentration of the salicyl to be somewhat less in the blood of rheumatic than nonrheumatic individuals.

PRESENCE OF FREE SALICYLIC ACID IN THE JOINT FLUID OF RHEUMATIC INDIVIDUALS

It has been claimed (notably by Binz,[3] Feri and others) that the action of salicylates in rheumatic fever is due to the liberation of free salicylic acid by carbon dioxid whose tension in the blood and other fluids is considered to be high enough for this in fever. We have tested this by direct extraction of freshly obtained specimens of joint fluid (from individuals suffering with rheumatic fever and receiving full therapeutic doses of salicylate) with ether, acetic ether and chloroform and then applying the ferric alum test to the ethereal extracts. By this method we have not been able to demonstrate the presence of free salicylic acid in all the fluids thus far examined (from ten patients—the five referred to in Table 1, and five others).

The ferric alum test is sensitive to about 1 : 1,000,000 of salicylic acid in 25 c.c. (0.000025 gm.) of solution. It is true that the total volume of synovial fluid obtained in each case was less than 25 c.c., but it must also be noted that in the smallest quantity of fluid (7 c.c.) there was about 32 times (0.0008 gram) and in the largest quantity (20 c.c.) there was about 140 times (0.0035 gram) as much total salicylate as the lowest limit of the test which permits the recognition of salicylic acid. In other words, there was considerable salicylate present, but not in the form of free salicylic acid. Any undetected free salicylic acid which might have been present could not be ascribed an important rôle as germicide or antiseptic if it is remembered that about 0.04 to 0.1 per cent. is necessary to inhibit the activity of enzymes,[4] and somewhat higher con-

3. Binz: Arch. f. exper. Path. u. Pharmakol., 1879, **10**, 147.
4. Kolbe: Jour. f. prakt. Chem., 1874, **10**, 89.

centrations (0.15 per cent.) to prevent the growth of, and about 0.35 per cent. to kill bacteria.[5] Objection might be raised to the method used because of the possibility that some CO_2 is lost when the fluid is exposed to the atmosphere, but this is to a considerable extent prevented by the presence of "buffer" or protective substances.

EFFECT ON THE ALKALI RESERVE AND REACTION OF BLOOD

The symptoms of "toxicity," i. e., deafness and ringing in the ears, nausea and vomiting produced by salicylate have been attributed by some to acidosis, that is, they are said to be due to or associated with acidity of the body fluids. Also it is a reason why sodium bicarbonate is commonly administered together with salicylate, i. e., to prevent the occurrence of acidosis. It is hardly conceivable that the true reaction (hydrogen ion concentration) of the blood could be sufficiently altered by salicylate so as to cause real acidity. The same might be said of the mechanism which maintains the reaction constant. However, this was tested out by observing the reaction and alkali reserve of the blood of individuals before, and after the administration of full therapeutic doses of sodium salicylate up to "toxicity." The reaction was estimated by the phenolsulphonephthalein-dialysis method of Levy, Rowntree and Marriott[6] and alkali reserve by the method of Marriott.[7] The results obtained are presented in Table 3. These show no noteworthy changes in the reaction and reserve alkalinity of the blood.

The same was found to be true of the blood of animals (cats and dogs) treated with the same, and, in some cases, even larger doses of salicylate per kilogram. The same methods were used and in some of the animals the blood was observed over long periods of time (three to nine days) after the administration of salicylate was stopped.

5. Bucholtz: Arch. f. exper. Path. u. Pharmakol., 1875, **4**, 1. Stockman: Brit. Med. Jour., 1913, **1**, 597.
6. Levy, Rowntree and Marriott: Arch. Int. Med., 1915, **16**, 389.
7. Marriott: Arch. Int. Med., 1916, **17**, 840.

TABLE 2.—CONCENTRATION OF SALICYL* IN BLOOD OF RHEUMATIC AND NONRHEUMATIC INDIVIDUALS

Nonrheumatic						Rheumatic				
			Blood					Blood		
Patient and Number	Diagnosis	Total Quantity Salicyl Administered, Gm.	Quantity Used, C.c.	Total Quantity Salicyl Recovered, Gm.	Concentration Salicyl, per Cent.	Patient and Number	Total Quantity Salicyl Administered, Gm.	Quantity Used, C.c.	Total Quantity Salicyl Recovered, Gm.	Concentration Salicyl, per Cent.
6 (F. D.)	T. B. kidney	12.04	17	0.006	0.035	L2 (M. E.)	7.83 31.3 2 days before	30	0.006	0.020
7 (L. E.)	Tabes	13.16	18	0.0058	0.032	L2 (H.)	12.29	13	0.0018	0.014
8 (M. H.)	Normal	15.48	10	0.0025	0.025	L4 (J. D.)	10.06	10	0.0016	0.016
10 (J. B.)	Normal	19.36	10	0.0038	0.038	1 (P. F.)	13.4	20	0.0031	0.016
13 (D. G.)	Normal	13.3	10	0.0023	0.023	2 (J. M.)	12.81	21	0.0016	0.008
16a (M. C.)†	Neph. Hys.	12.3	10	0.0018	0.018	3 (W. C.)	15.48	21.5	0.0061	0.028
16b (M. C.)	Neph. Hys.	11.4	15	0.0028	0.019	4 (H. J.)	14.45	20.4	0.006	0.030
19 (Y. V.)	T. B.	10.8	10	0.0024	0.024	5 (J. L.)	14.45	30.7	0.005	0.016
22 (E. C.)†	Surg.?	9.0	10	0.0024	0.024	8a (P. M.)	14.0	10	0.0023	0.023
						8b (M. M.)	4.5	20	0.006	0.030
						8a′(P. M.)	14.0	15	0.0023	0.015
						11 (S. V.)	17.00	10	0.0031	0.031
						12 (W. B.)	8.75	10	0.0025	0.025
						21 (J. V.)	10.8	10	0.0022	0.022
Average		13.0			0.0265		12.5			0.021

* Expressed as salicylic acid.

† Patient 16 had the following diagnosis diminished renal functional efficienty and hysteria: Patient 22 was a surgical case, diagnosis incomplete.

CONCLUSIONS

1. The percentage concentration of salicylate in the blood and joint fluid of rheumatic individuals receiving full therapeutic doses of the drug is approximately the same.

2. The concentration of salicylate tends to be less in the blood of rheumatic than nonrheumatic individuals.

3. There is no demonstrable free salicylic acid in the joint fluid of individuals suffering with rheumatic fever.

4. The true reaction and the reserve alkalinity of the blood are not perceptibly altered by the administration of salicylate even in large doses.

TABLE 3.—EFFECT OF SALICYL ON ALKALI RESERVE AND REACTION OF BLOOD*

Patient and Number	pH		RpH	
	B. S.	A. T.	B. S.	A. T.
21 (J. V.)	7.5	7.45	8.55	8.5
16a (M. C.)	7.45	7.45	8.4	8.4
16b (M. C.)†	7.75	7.7	8.55	8.5
17 (P. K.)†	7.45	7.5	8.55	8.5
18 (F. S.)	7.55	7.5	8.45	8.4
19 (Y. V.)	7.45	7.5	8.5	8.5
20 (G. W.)	7.5	7.5	8.4	8.4
22 (E. C.)	8.4	8.4	8.5	8.5
Mean	7.5	7.5	8.5	8.5

Animal and Number	pH		RpH		Body Weight, Kg.	Total Quantity Salicyl Administered, Gm.
	B. S.	A. T.	B. S.	A. T.		
Cat 8	7.4	7.4			1.9	0.85
Dog 11	7.6	7.7			6.3	2.19
		7.6				
		7.6				
Dog 12	7.7	8.0			5.5	3.5
Cat 13	7.6	7.6	8.2	8.2	2.1	0.94
Dog 17	7.8	7.7	8.4	8.2	7.5	3.0
		7.8		8.2		
		7.8		8.2		
		7.5		8.5		
		7.7		8.4		
		7.6		8.2		
		7.6		8.4		
		7.6		8.6		
		7.6		8.4		

* For other data on the patients in this table see Table 2 and p. 34; all salicyl quantities are expressed as salicylic acid. pH refers to hydrogen ion concentration (reaction) and RpH to alkali reserve of the blood; B. S. = before salicylate; A. T. = at "toxicity" and thereafter.

†At the end of the experiment the results for 16b were; pH = 7.5, RpH = 8.45; for 17, pH = 7.45, RpH = 8.45.

THE SALICYLATES

V. EXCRETION OF SALICYL IN THE URINES OF RHEUMATIC AND NONRHEUMATIC INDIVIDUALS *

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AND

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Various explanations have been offered for the striking action of salicylates in acute articular rheumatism. None of these, however, has been satisfactorily established. We have investigated the subject along several lines, but are not prepared to support any explanation. The object of this contribution is to compare the quantitative excretion of salicyl in rheumatic and nonrheumatic, or practically normal, subjects. This was intended to show whether the therapeutic effects of salicylate could be based on differences in the retention or "affinity" of the normal and rheumatic organism for salicyl. The excretion in a number of individuals suffering with various clinical conditions other than rheumatism was also studied.

The work was conducted in a quantitative manner as much as possible. A definite quantity (20 c.c.) of a solution of sodium salicylate of known strength (averaging about 10 per cent.) was administered every hour until signs or symptoms of "toxicity" appeared, when the administration was stopped. The average "toxic" dose was about 13.8 grams, expressed

* This investigation was supported in part by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

as salicylic acid. The fluid intake was maintained as nearly constant as possible, that is, usually 100 c.c. every hour (20 c.c. salicyl solution and 80 c.c. water) until "toxicity," then 200 c.c. every two hours until the experiment was terminated. Urine was collected in fractional specimens every ten hours throughout the experiment and until salicyl ceased to be excreted. This lasted usually three to four days. In the meantime careful observations were made of all functions and symptoms such as respiration, blood pressure, pulse rate, temperature, etc. The salicyl content of each urine specimen was determined in duplicate by the distillation-colorimetric method,[1] and in addition each specimen of urine was also studied for the presence of albumin, casts, blood, acetone and diacetic acid. Blood was secured at different stages of the experiment for nonprotein and urea nitrogen estimations, and the phenolsulphonephthalein test for renal function was also made. A preliminary report[2] of the studies on renal function has been made; the full report is soon to follow. In this paper reference will be made only to the urinary excretion of salicyl. The results here reported have been obtained from a series of twenty-four experiments on twenty different individuals. Six of these were nonrheumatic and practically normal individuals; eight (inclusive of 21a, who is the same as 21b among the normals) suffered with rheumatic fever, and seven (Patient 16 appearing four times) with other clinical conditions. The various clinical and related data pertaining to all the individuals are summarized in Table 1.

I. LESSENED TOTAL EXCRETION IN RHEUMATIC FEVER

As indicated by the data in Table 2, which is intended as a comparative summary of the results of all the experiments, the total excretion of salicyl is about 15 per cent. less in rheumatic than normal individuals. That is, about 75 per cent. is excreted by

1. Thoburn and Hanzlik: Jour. Biol. Chem., 1915, **23**, 163.
2. Scott and Hanzlik: Jour. Am. Med. Assn., 1916, **67**, 1838.

TABLE 1.—CLINICAL AND OTHER DATA

No. and Patient	Diagnosis	Condition of Temperature	Fluid Intake	Total Quantity of Salicyl[5] Administered, Gm.	Remarks
			Normal Individuals		
6 (F. D.)	Normal[1]	Afebrile	180 c.c. every hour for 7 hours 300 c.c. every 2 hours thereafter	12.04	
7 (L. E.)	Tabes[2]	Afebrile	150 c.c. every hour for 8 hours 300 c.c. every 2 hours thereafter	13.76	
8 (M. H.)	Normal	Afebrile	150 c.c. every hour for 9 hours 400 c.c. every 2 hours thereafter	15.48	
9 (H. P.)	Normal	Afebrile	100 c.c. every hour for 12 hours 200 c.c. every 2 hours thereafter	14.96	
21b (J. V.)	Normal[3]	Afebrile	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter	9.5	
P. J. H.	Normal	Afebrile	Irregular	5.7[4]	
Average				13.2	
			Rheumatic Individuals		
1 (P. F.)	R. F.	Febrile	256 c.c. every hour for 12 hours 256 c.c. every 2 hours thereafter	13.41	
2 (J. M.)	R. F.	Febrile[7]	170 c.c. every hour for 8 hours 300 c.c. every 2 hours thereafter	12.81	
3 (W. C.)	R. F.	Febrile[7]	100 c.c. every hour for 9 hours 200 c.c. every 2 hours thereafter	15.48	
4 (J. H.)	R. F.	Febrile	220 c.c. every hour for 8 hours 440 c.c. every 2 hours thereafter	14.45	
5 (J. L.)	R. F.	Febrile	200 c.c. every hour for 8 hours 400 c.c. every 2 hours thereafter	14.45	
11 (S. V.)	R. F.	Febrile	100 c.c. every hour for 11 hours 200 c.c. every 2 hours thereafter	17.00	
12 (W. B.)	R. F.	Febrile[7]	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter	8.75	
21a (J. V.)	R. F.	Febrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	10.20	
Average				13.9	

Miscellaneous Conditions

10 (J. B.)	Chronic alcoholism	Afebrile	100 c.c. every hour for 11 hours 200 c.c. every 2 hours thereafter	19.39	
16a (M. C.)	Hys. Neph.[5]	Afebrile	100 c.c. every hour for 7 hours 200 c.c. every 2 hours thereafter	12.30	
16b (M. C.)	Hys. Neph.	Afebrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	11.49	12 grams $NaHCO_3$ administered together with salicyl
16c (M. C.)	Hys. Neph.	Afebrile	100 c.c. every hour for 7 hours 200 c.c. every 2 hours thereafter	10.50	
16d (M. C.)	Hys. Neph.	Afebrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	9.36	
17 (P. K.)	Chronic alcoholism	Afebrile[8]	100 c.c. every hour for 7 hours 200 c.c. every 2 hours thereafter	12.30	14 grams $NaHCO_3$ administered together with salicyl
18 (F. S.)	Chronic alcoholism	Afebrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	10.80	
19 (Y. V.)	Tuberculosis	Very febrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	10.80	
20 (G. W.)	Chronic morphinism	Afebrile	100 c.c. every hour for 6 hours 200 c.c. every 2 hours thereafter	10.80	
22 (E. L.)	Surg.[6]	Afebrile	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter	9.0	
Average				11.1	

1. Three months before the salicyl experiment this patient was operated for appendicitis; phenolsulphonephthalein excretion 60 per cent. in two hours, on three different occasions; later, tuberculosis of kidney suspected, but not demonstrated; considered practically normal.

2. Blood and spinal fluid gave positive Wassermann tests; loosing control of legs, but otherwise well; received antisyphilitic treatment; improved; afebrile; at time of salicyl experiment considered practically normal.

3. Same as Patient 21a; one month before, had an attack of rheumatic fever and was successfully treated with salicylate; patient became well and was about to be discharged when Experiment 21b was performed, and at this time was considered practically normal.

4. Not a "toxic" dose, and was excluded from the average.

5. This patient showed a two-hour phenolsulphonephthalein excretion of 25, 35 and 35 per cent., on three different occasions, respectively, during an interval of about four months. The diagnosis was hysteria and diminished renal functional efficiency.

6. A surgical condition, possibly renal; diagnosis incomplete.

7. The fever in these individuals was not severe, the temperature in each case not reaching over 100.4 F.

8. Partially febrile, temperature reaching a maximum of 98.8 F.

9. Expressed as salicylic acid.

TABLE 2.—SUMMARY OF TOTAL PERCENTAGE EXCRETION OF SALICYL * IN THE URINES OF DIFFERENT INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF THE DRUG

Normal		Rheumatic		Miscellaneous Conditions		
No.	Salicyl Excreted, Per Cent.	No.	Salicyl Excreted, Per Cent.	No.	Diagnosis	Salicyl Excreted, Per Cent.
6	91.9	1	45.0	10	Chronic alcoholism	45.8
7	72.4	2	71.7	16a	Neph./Hys.	63.7
8	77.3	3	73.7	16b	Neph./Hys.	55.1
9	71.7	4	59.5	16c	Neph./Hys.	53.6
21b	72.2	5	61.3	16d	Neph./Hys.	60.4
P. J. H.	78.1	11	54.9	17	Chronic alcoholism	63.1
		12	65.0	18	Chronic alcoholism	58.0
		21a	57.7	19	Tuberculosis	52.9
				20	Chronic morphinism	63.2
				22	Surg.?	57.7
Median	75.0		60.4			57.8

* Expressed as salicylic acid.

TABLE 3.—PERCENTAGE EXCRETION OF SALICYL * AT THE END OF EACH TEN-HOUR PERIOD BY RHEUMATIC AND NORMAL INDIVIDUALS

Number	Per Cent. of Salicyl Excreted End of a Ten-Hour Period										
	1	2	3	4	5	6	7	8	9	10	11
Normal Individuals											
6	49.3	62.5	71.7	79.8	85.6	90.1	91.7	91.9			
7	25.1	37.3	44.6	53.2	60.3	66.5	69.2	72.1	72.4		
8	31.1	41.8	52.3	61.0	70.5	74.6	77.1	77.3			
9	6.1	14.1	38.8	48.9	60.0	63.9	70.1	71.2	71.7		
21b	18.6	44.5	53.2	63.6	68.4	71.2	72.1	72.2			
Median	25.1	41.8	52.3	61.0	68.4	71.2	72.1	72.2	72.0		
Rheumatic Individuals											
1	5.5	19.3	27.2	33.2	40.1	43.8	45.0				
2	11.6	33.7	43.3	53.1	60.4	66.7	70.7	71.7			
3	17.2	33.3	42.1	49.6	58.3	63.7	69.1	72.4	73.5	73.7	
4	15.0	29.5	36.5	42.3	48.1	52.3	55.9	58.0	59.0	59.4	59.5
5	22.9	34.0	41.5	48.3	52.9	57.9	61.2	61.3	61.3		
11	5.8	15.1	18.2	27.6	33.6	39.7	44.8	49.6	52.4	53.9	54.9
12	14.1	23.7	39.4	48.7	56.3	63.5	64.8	64.9	65.0	65.0	
21a	6.0	14.8	24.3	35.0	44.4	51.6	56.7	57.6	57.7		
Median	12.9	26.6	38.0	45.3	50.5	55.6	59.0	61.3	60.2	62.2	

* Expressed as salicylic acid.

normal, and about 60 per cent. by rheumatic individuals. The general course of the excretion at the end of each ten-hour period is further indicated by the curves in Figure 1, constructed from the data in Table 3, which contains the results of all the experiments except Experiment "P. J. H.," in which the excretion

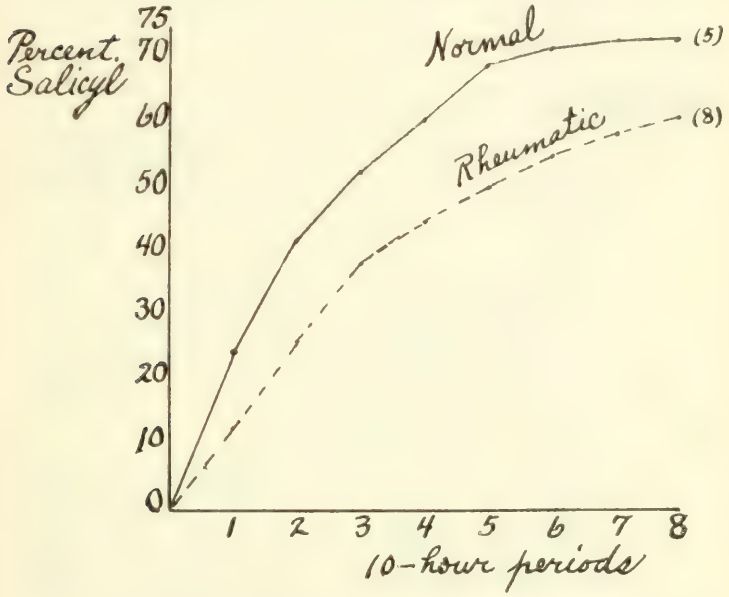

Fig. 1.—Percentage of excretion of salicyl at the end of each ten-hour period by rheumatic and normal individuals. The curves represent median values; the number in parentheses, number of individuals.

was not fractionated. The results obtained with individuals suffering with other clinical conditions will be referred to later.

By means of the ether extraction method Feser and Friedberger[3] observed that about 63 per cent. of the total salicyl is excreted in urine. Applying the colorimetric method directly to alcoholic extracts of salicyl

3. Feser and Friedberger: Arch. f. wissensch. u. prakt. Tierheilk., 1875, H. 2, 3, and 6, Berl. klin. Wchnschr., 1875, **12**, 321.

urines, Wiley[4] found the total excretion to be about 46.8 per cent. On the other hand, U. Mosso[5] claimed the total excretion to be from 96.8 to 106.7 per cent. However, the accuracy of Mosso's gravimetric method, which was long and involved, is open to some doubt. Our results, which represent total salicyl in whatever form it might exist in urine (i. e., conju-

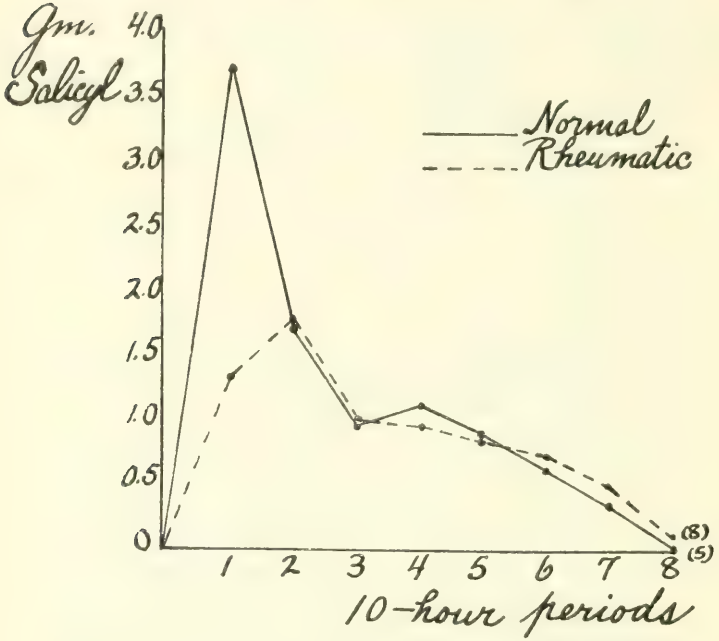

Fig. 2.—Total quantity of salicyl excreted in each ten-hour period by rheumatic and normal individuals. The curves represent median values; the figures in parentheses, number of individuals.

gated or free, or both), while somewhat higher, nevertheless tend to support those of Feser and Friedberger and Wiley. They also show conclusively that salicyl is not excreted to as great an extent as has been hitherto generally supposed.

4. Wiley: U. S. Dept. Agric. Bur. Chem. Bull., No. 84, Part II, 1906.
5. Mosso, U.: Arch. f. exper. Path. u. Pharmakol., 1889, **26**, 267.

II. EXCRETION IN THE EARLY PERIODS AFTER ADMINISTRATION OF SALICYL

The difference in excretion is greatest in the early periods, that is, during the first ten to twenty hours after administration of the salicylate. This is illustrated by Figure 2, which represents the median quantity of salicyl excreted in each ten-hour period by normal and rheumatic individuals. The data for these are presented in Table 4.

TABLE 4.—TOTAL QUANTITY OF SALICYL* EXCRETED IN EACH TEN-HOUR PERIOD BY RHEUMATIC AND NORMAL INDIVIDUALS

Number	Grams of Salicyl Excreted in a Ten-Hour Period										
	1	2	3	4	5	6	7	8	9	10	11
Normal Individuals											
6	5.936	1.590	1.107	0.974	0.712	0.539	0.191	0.021			
7	3.440	1.690	1.028	1.167	0.975	0.854	0.374	0.395	0.038		
8	4.822	1.655	1.631	1.333	1.486	0.624	0.392	0.027			
9	3.700	1.196	0.907	1.518	1.656	0.575	0.932	0.242	0.005		
21b	1.766	2.465	0.828	0.980	0.458	0.266	0.087	0.006			
Median	3.700	1.655	1.028	1.167	0.975	0.624	0.374	0.027	0.022		
Rheumatic Individuals											
1	0.740	1.848	1.058	0.804	0.932	0.489	0.158				
2	1.484	2.830	1.238	1.244	0.935	0.717	0.605	0.124			
3	2.675	2.479	1.366	1.157	1.357	0.830	0.832	0.523	0.171	0.020	
4	2.167	2.103	1.004	0.850	0.826	0.600	0.535	0.300	0.150	0.055	0.007
5	3.305	1.613	1.078	0.984	0.664	0.722	0.478	0.010	0.009		
11	0.989	1.579	0.532	1.584	1.021	1.050	0.864	0.810	0.480	0.250	0.180
12	1.229	0.843	1.373	0.616	0.662	0.633	0.116	0.009	0.003		
21a	0.608	0.898	0.973	1.088	0.950	0.732	0.525	0.088	0.009		
Median	1.357	1.731	1.068	1.033	0.933	0.722	0.530	0.124	0.150		

* Expressed as salicylic acid.

III. CONCENTRATION OF SALICYL IN BLOOD AND URINE IS LESS IN RHEUMATIC INDIVIDUALS

In a previous paper[6] it was shown that the concentration of salicyl in the blood of rheumatic individuals at "toxicity" (about the end of eight to ten hours after administration) tends to be less than in normal individuals. The average concentration for normal

6. Scott, Thoburn and Hanzlik: Jour. Pharmacol. and Exper. Therap., 1917, **9**, 217.

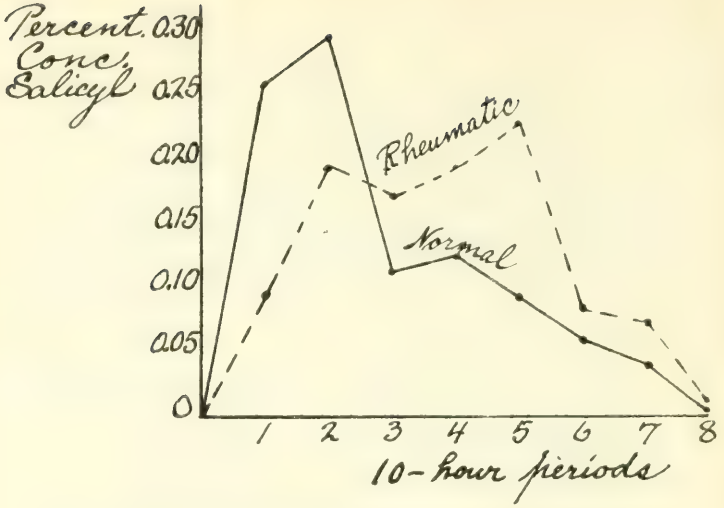

Fig. 3.—Percentage concentration of salicyl in the urines of normal and rheumatic individuals. The curves represent median values.

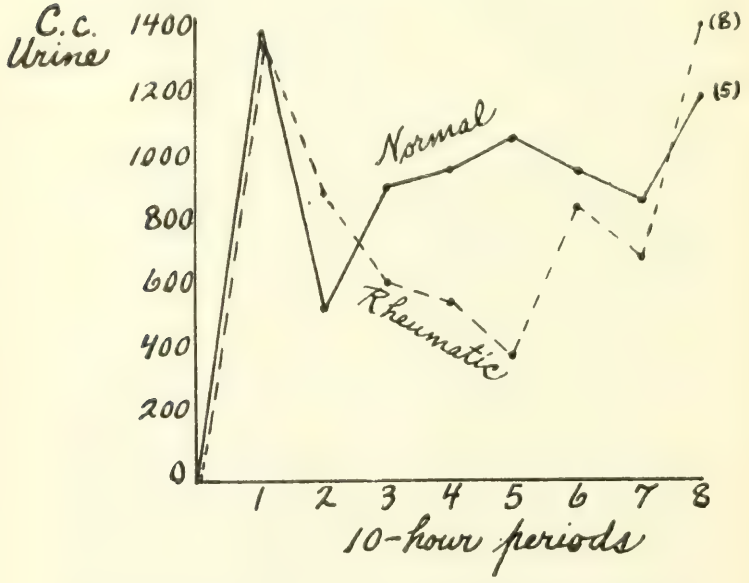

Fig. 4.—Excretion of urine in each ten-hour period following the administration of full therapeutic doses of salicyl to normal and rheumatic individuals. The curves represent median values; the figures in parentheses, number of individuals.

individuals was found to be 0.0265 per cent.; for rheumatics, 0.021 per cent. The median percentage concentration of salicyl in the urine is also less in rheumatic than normal individuals. This is illustrated by the curves in Figure 3, which were constructed from the data in Tables 4 and 5.

TABLE 5.—EXCRETION OF URINE IN EACH TEN-HOUR PERIOD FOLLOWING THE ADMINISTRATION OF FULL THERAPEUTIC DOSES OF SALICYLATE TO NORMAL AND RHEUMATIC INDIVIDUALS

Number	Cubic Centimeters of Urine in Each Ten-Hour Period										
	1	2	3	4	5	6	7	8	9	10	11
Normal Individuals											
6	3230	467	1315	899	1071	978	708	1472			
7	1405	1390	925	1092	1333	1088	875	1706			
8	1970	786	1359	1519	1085	1478	1502	2264	1828		
9	810	550	625	230	360	185	630	755	1027		
21b	1380	425	250	980	730	950	1450	700	1760		
Median	1405	550	925	980	1071	978	875	1206	1760		
Rheumatic Individuals											
1	1720	1123	703	428	404	1060	1858				
2	1763	2322	754	814	1504	1066	1840	1433			
3	2001	446	320	274	343	325	628	943	864	791	
4	3153	1094	1307	1884	1586	1586	2080	1642	2144	2073	1443
5	1074	717	579	634	400	662	464	1491	1191		
11	595	420	175	495	290	263	263	455	375	387	392
12	640	340	660	680	630	1120	775	1170	960		
21a	950	340	320	340	235	380	525	400	315		
Median	1397	906	620	565	402	861	707	1420	927		

IV. EXCRETION IS UNINFLUENCED BY DIURESIS

The differences in total excretion and concentration are not due to incomplete collection, for in each case the urine was collected until salicyl-free. Furthermore, the diuresis during the early periods, that is, the first ten to twenty hours after administration of the drug, was practically the same in both rheumatic and normal individuals. This is indicated by the data in Table 5 and the curves in Figure 4.

V. RETENTION OF SALICYL

This might explain the differences in the urinary excretion and concentration of salicyl. However, it is believed our data do not indicate there is any. The following results are urged for this contention: (1) The duration of the excretion, as indicated by certain data in Table 6, was practically the same in both rheu-

TABLE 6. DURATION OF SALICYL EXCRETION IN THE URINES OF NORMAL, RHEUMATIC AND OTHER INDIVIDUALS

Normal		Rheumatic		Miscellaneous Conditions	
No.	Hours	No.	Hours	No.	Hours
6	74	1	68	10	100+
7	76	2	72	16a	80
8	84	3	93	16b	80+ft. tr.
9	80	4	102	16c	90+
21b	80	5	70	16d	90
P. J. H.	80	11	110+	17	90+ft. tr.
		12	90	18	110+ft. tr.
		21a	100+	19	110
				20	100+ft. tr.
				22	100
Median	78 (3¼ days)*		72 (3 days)†		

* From Nos. 6, 7, 8 and P. J. H., which represent two-hour collections of urine.

† From Nos. 1, 2, 3, 4 and 5 which represent two-hour collections of urine. In all other cases in this table ten-hour collections were made throughout. + means that a trace of salicyl was present in the next ten-hour specimen of urine, the exact cessation of excretion in such a specimen, therefore, not being known; ft. tr., means a faint trace (almost questionable) of salicyl was present in the next ten-hour specimen.

matic and normal individuals, that is, seventy-two hours (three days), and seventy-eight hours (three and one-fourth days), respectively. These results are drawn from Experiments 1, 2, 3, 4 and 5 for the rheumatic, and 6, 7, 8, and P. J. H., for the normal individuals, since, in these, the urines were collected every two hours toward the end of the experiment and represent, therefore, more precisely the cessation of excretion than in the other experiments in which ten-hour collections only were made.

The duration of excretion depends to a certain extent on the dosage. After doses of 1 to 2 grams of salicylate, Blanchier[7] found excretion to be com-

7. Blanchier: Thése de Paris, No. 141.

pleted in twenty-two hours; after 4 to 5 grams, at the end of forty-four hours. Blanchier also claimed that in rheumatic individuals (doses of 4 to 8 grams) traces of salicyl in urine were found at the end of seventy-two hours. Other claims concerning the completion of elimination have been made as follows: Drasche,[8] five days, after ordinary therapeutic doses; Geissler,[9] twelve hours; Sée,[10] twenty-four to forty-eight hours; Fleischer,[11] thirty-six hours; Hutinel,[12] nineteen to twenty-eight hours, using small doses (0.5 gram approximately) of salicylate; Ehrmann,[13] who found the excretion to last thirty-six to forty-eight hours in both normal and rheumatic individuals.

(2) The concentration of salicyl in the blood is less in rheumatic than normal individuals.

VI. VICARIOUS EXCRETION OF SALICYL

As to excretion by channels other than urine the results presented in Table 7 were obtained.

Feces. Salicyl was recovered from the feces by a method previously described. Collections over three to four days, that is, until the termination of the experiment, were obtained from six individuals. These showed practically only a trace, that is, from none to 0.07 gram of salicyl present, or from none to about 0.52 per cent. of the total quantity administered. This shows that the absorption of salicylate from the alimentary canal is very complete, the loss by the feces being negligible.

Sweat. It is possible that excretion by the sweat might explain, in part at least, the loss of salicyl in the urine, particularly since a number of the individuals exhibited rather profuse diaphoresis following the administration of the drug. The method used in attempting to ascertain the quantity of salicyl present was not ideal. However, it is believed any consider-

8. Drasche: Wien. med. Wchnschr., 1876, **26**, 1049.
9. Geissler: Gaz. méd., 1877, Nos. 7 and 8.
10. See: Bull. de l'Acad. de méd., 1877, **6**, 689.
11. Fleischer: Berl. klin. Wchnschr., 1875, **12**, 529.
12. Hutinel: Thése de Paris, 1913, No. 354, p. 52.
13. Ehrmann: München. med. Wchnschr., 1907, **54**, 2595.

able quantity, such as a gram, for instance, could be detected, the discrepancy in the excretion in the majority of individuals being greater than this.

The salicyl was recovered by thoroughly washing and rinsing all of the garments worn by the patient and bedclothes (except mattress) after the experiment was terminated in 20 to 25 liters of alkaline water and to this was added the soapy bath-water from the patient. The washings were evaporated on a water-bath to a convenient volume (100 c.c. or so),

TABLE 7.—RECOVERY OF SALICYL* FROM FECES AND SWEAT

No.	Total Quantity of Salicyl Administered, Gm.	Feces			Sweat	
		Total Quantity, Gm.	Total Quantity of Salicyl Recovered, Gm.	Per Cent. of Total Administered	Total Quantity of Salicyl Recovered, Gm.	Per Cent. of Total Administered
1	13.41	420	0.070	0.52		
2	12.81	1,960	0.044	0.34		
3	15.48	2,200	0.059	0.38		
4	14.45	320	0.010	0.07		
7	13.76	50	None	None		
8	15.48	2,200	0.004	0.026		
10	19.30				0.002†	0.010
11	17.00				0.017‡	0.100
17	12.30				0.0014	0.011
18	10.80				0.0022	0.020
19	10.80				0.004	0.038
20	10.80				0.0022	0.020

* Expressed as salicylic acid.

† Required an addition of 0.5 c.c. of 5 per cent. phenol (0.025 gram) to the standard to match the color of the distillate from this sweat.

‡ Required an addition of 0.5 c.c. of 5 per cent. phenol (0.025 gram) to the standard to match the color of the distillate from this sweat. All other distillates matched very well with our salicylate standard.

acidified, distilled, and estimated for salicyl colorimetrically in the usual way. The quantities recovered ranged from 0.002 to 0.017 gram, or about 0.01 to 0.1 per cent. of the total administered, and may be regarded as practically negligible.

It is seen, therefore, that the losses of salicyl by both the feces and sweat are too small to account for the difference in the urinary excretion between normal and rheumatic individuals. It is hardly conceivable that sufficient loss of salicylate could occur by evaporation from the skin.

VII. DESTRUCTION OF THE SALICYL

Having eliminated the influence of such factors as diuresis, retention and vicarious excretion, it seems logical to conclude that the difference in the excretion of salicyl between normal and rheumatic individuals is due to destruction of the salicyl. Not all the salicyl is recoverable from the urines of normal individuals. In other words, there is some destruction, though variable, even in normal individuals. It has been shown that other aromatic derivatives related to salicylate are destroyed in the living organism. Seidell[14] recovered only 31 to 46 per cent. of thymol (according to the dosage) in the urine of dogs, and concluded that the remainder was destroyed or temporarily fixed in the body. According to Dubin[15] the administration of phenol and p-cresol to dogs gave a recovery of 65 and 40 per cent., respectively, as phenols. Tauber[16] concluded that a fraction of administered phenol is burned, for he recovered only about 50 per cent. in the urine and only traces in the feces. After the administration of 0.1 gm. of phenol de Jonge[17] recovered 78 per cent. of the phenol in his urine. Jonescu[18] found that only 25 per cent. of p-cresol fed to dogs was excreted. Conversion of benzene (C_6H_6) in dogs and rabbits to muconic acid to the extent of about 0.3 per cent., and with an improved method of analysis 25 per cent., is reported by Jaffé.[19] Finally, Dixon[20] states that salicylates are changed chemically in the liver. However, the evidence for this is not cited.

It would hardly be possible to draw a precise relationship between the severity of the fever and percentage excretion of salicyl in the urine. However, it must be noted that, in general, the excretion was better in the afebrile than febrile individuals. Also,

14. Seidell: U. S. Hygienic Lab. Bull., 101, 1915, p. 43.
15. Dubin: Jour. Biol. Chem., 1916, **26**, 69.
16. Tauber: Z. physiol. Chem., 1878-1879, **2**, 366.
17. De Jonge: Ibid., 1897, **3**, 181.
18. Jonescu: Biochem. Zeit., 1906, **1**, 399.
19. Jaffé: Z. physiol. Chem., 1909, **62**, 58.
20. Dixon: Manual of Pharmacology, 1915, p. 39. Arnold, London.

in two of the febrile rheumatic individuals, namely, Patients 2 and 3, the fever was less severe than in the other rheumatics and the excretion was better. A still more striking illustration of the tendency of excretion in fever are the results from Patient 21. While suffering with an attack of acute rheumatic fever, the total excretion of salicylate by this patient (21a) was 57.7 per cent. and after he became well (21b), that is, afebrile, etc., he could excrete 72.2 per cent. of the total ingested salicylate. In other words, the excretion, when the individual was afebrile, was practically normal, and certainly much improved.

So far as renal functional efficiency is concerned, this was generally quite as good in rheumatic as in the normal individuals. This ranged from 55 to 75 per cent. for the phenolsulphonephthalein excretion for both normal and rheumatic individuals. The non-protein nitrogen of the blood ranged from 24 to 53.1 mg. and 31.1 to 66.3 mg. (in 100 c.c.) for the normal and rheumatic individuals, respectively. So far as the effect of salicylate on renal function is concerned this was generally in the same direction in both types of individuals, namely, a diminution, although there was some slight variation in this. In general there seemed to be no constant relationship between the renal functional efficiency in these individuals and the excretion of salicylate to account for the difference in excretion between the normals and rheumatics. It is, of course, conceivable that with a marked diminution in renal functional efficiency to begin with the salicyl would tend to be retained and subjected longer to the destructive action of the tissues. Such, for instance, might conceivably be the case in Patient 16. This will be referred to in the next section.

Taking all of the various evidences into consideration it appears conceivable that a greater destruction of salicyl occurs in the febrile rheumatic than normal organism. As yet it would be idle to speculate as to the nature or reason of this destruction.

VIII. EXCRETION OF SALICYL IN VARIOUS CLINICAL CONDITIONS

The conception of increased destruction of salicyl in the febrile rheumatic organism led to the study of the total excretion in other fevers and conditions such as chronic morphinism and alcoholism in which there is said to be an increased destruction of the drug to which the individual is habituated. Thus far a variety of four conditions has been studied, namely, chronic alcoholism, chronic morphinism, tuberculosis (fever) and lessened renal functional efficiency. In one patient the experiment was repeated four times,

TABLE 8. TOTAL QUANTITY OF SALICYL* EXCRETED IN EACH TEN HOUR PERIOD BY INDIVIDUALS SUFFERING WITH VARIOUS CLINICAL CONDITIONS†

No.	Grams of Salicyl Excreted in a Ten-Hour Period										
	1	2	3	4	5	6	7	8	9	10	11
10	1.710	1.344	2.040	1.308	0.709	0.729	0.350	0.407	0.231	0.027	
16a	2.632	1.188	0.354	1.600	0.658	0.272	0.569	0.566			
16b	3.040	1.050	0.412	0.749	0.424	0.265	0.282	0.055			
16c	1.280	1.578	0.425	0.391	1.305	0.269	0.363	0.014	0.007	0.001	
16d	1.398	0.623	0.938		1.473	0.283		0.830	0.106		
17	3.350	2.174	1.026	0.760	0.405	0.014	0.014	0.001	0.001		
18	1.725	0.990	0.843	0.600	0.913	1.103	0.024	0.049	0.012	0.004	
19	1.254	1.408	0.951	0.678	0.585	0.500	0.242	0.076	0.008	0.014	0.002
20	1.778	1.325	1.339	0.954	0.713	0.652	0.014	0.030	0.004	0.013	
22	0.471	1.908	0.998	0.728	0.589	0.419	0.041	0.025	0.006	0.004	
Median	1.713	1.335	0.945	0.749	0.683	0.351	0.242	0.052	0.007	0.008	

* Expressed as salicylic acid.
† For diagnoses see Table 1.

the results from all agreeing very closely. The data from all the ten experiments are presented in Table 8 (excretory results) and Table 9 (diuresis), and, in addition, a summary is included in Table 2. These show a range of excretion from 45.8 to 63.7 per cent. with a median value of 57.8 per cent., approaching closely, therefore, the excretion in rheumatic individuals, but being considerably less than in normal individuals.

These facts seem to give support to the conception of increased oxidation or destruction in these conditions. It is hoped, however, to extend the observations to other conditions more definitely fulfilling the

requirements of this conception, and on a greater number of individuals, before definite conclusions are drawn.

IX. EFFECT OF BICARBONATE ON THE EXCRETION OF SALICYL

Fleischer[11] has made the claim that the administration of sodium bicarbonate shortens the period of elimination of salicylate from thirty-six to fifteen hours. Also, according to Ehrmann[12] the administration of alkali hastens the excretion of salicyl. It may not be out of place here to call attention to another claim that has been made for bicarbonate, namely, Lees[21] states that the administration of sodium bicarbonate with salicylate modifies or prevents the appearance of symptoms of "toxicity," and even convulsions, but according to Meara[22] the use of alkalies together with salicylates has been dictated more by tradition than by any rationale.

TABLE 9.—EXCRETION OF URINE IN EACH TEN-HOUR PERIOD FOLLOWNG THE ADMINISTRATION OF FULL THERAPEUTIC DOSES OF SALICYLATE TO INDIVIDUALS SUFFERING WITH VARIOUS CLINICAL CONDITIONS *

No.	Cubic Centimeters of Urine in Each Ten-Hour Period									
	1	2	3	4	5	6	7	8	9	10
10	595	420	175	495	290	263	263	455	375	
16a	1,645	270	145	400	412	400	395	395		
16b	1,510	375	122	360	655	575	440	962	338	558
16c	1,230	290	127	115	290	119	275	760	110	105
16d	760	205	350		1,315	505		2,305	1,510	
17	1,015	155	270	380	750	460	310	400	430	430
18	1,150	225	170	150	250	540	339	365	410	590
19	1,105	352	320	265	220	305	505	860	1,030	510
20	1,140	250	270	310	230	1,165	154	740	410	475
22	1,425	1,070	450	640	910	920	930	1,140	1,560	1,100
Median	1,145	321	223	360	351	483	339	750	410	510

* For diagnoses see Table 1.

So far as the effect on the quantity and duration of excretion of salicyl is concerned the results obtained by us do not support the claims that have been made. This is illustrated by the results obtained with Patients 16c and 17, who received bicarbonate together with salicylate in equal dosage. The "toxic"

21. Lees: Proc. Roy. Soc. Med. (Pharm. Sec.), 1908-1909, **2,** 34.
22. Meara: Am. Jour. Med. Sc., 1910, **139,** 328.

dose was practically no greater than the dosage without bicarbonate in the same (Patient 16a, b and d) and other individuals of the corresponding group. The duration of excretion was about the same and the quantity of salicyl eliminated also. In the case of Patient 16 there are three controls, and Patient 17 may be compared with Patient 16 and others of his group. The usual symptoms of "toxicity" appeared in these patients. As a matter of fact there are records of several hundred patients in the Lakeside and City Hospitals of Cleveland having been made "toxic" by the routine administration of salicylate together with bicarbonate as a therapeutic procedure in the treatment of rheumatic fever.

Bicarbonate, therefore, has no demonstrable influence on the quantity and duration of excretion of salicyl and the "toxic" effects produced by the drug.

X. CONCLUSIONS

1. The total excretion of salicyl is about 15 per cent. less in rheumatic than normal individuals.

2. This difference is greatest in the early periods after the administration of the drug, that is, during the first ten to twenty hours.

3. The concentration of salicyl in the blood of rheumatic individuals at "toxicity" is less, and the concentration in the urine at this time is also less than in normal individuals.

4. These differences are not due to diuresis; nor to retention and vicarious excretion of the salicyl.

5. They seem to be due to an increased destruction of the salicyl in the febrile rheumatic organism.

6. A lessened excretion of salicyl was demonstrated in a number of individuals representing such conditions as chronic alcoholism and morphinism, tuberculosis and diminished renal functional efficiency as compared with normal individuals.

7. The administration of sodium bicarbonate together with the salicylate has practically no effect on the excretion of salicyl in the urine, or its "toxic" effects.

THE SALICYLATES

VI. RENAL, FUNCTIONAL AND MORPHOLOGIC CHANGES IN ANIMALS FOLLOWING THE ADMINISTRATION OF SALICYLATE *

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(*From Archives of Internal Medicine, June, 1917*)

It was previously shown[1] that individuals receiving full therapeutic doses of salicylate invariably have albumin, leukocytes and casts or cast-like bodies in the urine. This albuminuria is produced in both febrile and afebrile individuals, and is of renal origin. The functional tests (penolsulphonephthalein excretion in urine and nonprotein nitrogen of the blood) which were applied at the time, gave inconstant results, and the changes in some cases were so small that it was impossible to conclude definitely whether any serious impairment in the functional efficiency of the kidney existed. It is possible that repeated administration of salicylate might cause more definite and marked changes in the kidney, both functionally and morphologically. Moreover, in certain individuals, the albuminuria and other changes are so severe that the presence of a definite nephritis is suggestive.

Von Ackeren[2] gave sodium salicylate and salicylic acid per os to five rabbits, and two of these animals

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* This investigation was supported in part by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Scott, R. W., and Hanzlik, P. J.: The Salicylates: III. Salicylate Albuminuria, Jour. Am. Med. Assn., 1916, **67,** 1838.

2. Von Ackeren, A.: Ueber Nierenreizung nach Salicylsäure Gebrauch, Charité Ann., 1890, **15,** 252.

showed "acute nephritis," with albuminuria and hematuria. He cites fourteen cases in the literature up to 1890, in which there was albuminuria, sometimes accompanied by hematuria, following administration of salicylate. Vinci[3] observed a toxic parenchymatous nephritis at the necropsy of a man 45 years of age, who died thirty-one hours after the ingestion of 35 gm. of sodium salicylate. There was marked congestion of all the renal vessels, particularly in the glomerular tuft, albuminous precipitate in the subcapsular space, interstitial hemorrhage, cloudy swelling and extensive necrosis of the tubular epithelium, most marked in the cortex; but as the necropsy was performed two days after death, June 25, and as the kidneys sent to Vinci were imperfectly preserved in alcohol, little importance can be attached to the histologic findings. Experimenting with dogs, guinea-pigs and rabbits, he found that massive single doses, and frequently repeated large doses, led essentially to the same change as he described in the human kidney. For the most part the drug was given by mouth, but in a few instances was given intravenously or subcutaneously. He states that the lesions were most marked in dogs, less in guinea-pigs and least in rabbits. Lüthje,[4] in addition to a large amount of clinical work, gave repeated doses of sodium salicylate to two dogs, and both showed cylindruria, one showing albuminuria. Busse examined the kidneys and found general congestion, interstitial hemorrhage, cloudy swelling, fatty degeneration, cast formation, and in the subcapsular space of the glomerulus a homogeneous coagulum. With the exception of the reports of von Ackeren, Lüthje and Vinci, the literature seems to be devoid of carefully controlled experimental work on the effect of salicylate on the kidneys of lower animals.

3. Vinci, G.: Sulle lesioni istologiche sperimentali del rene determinate dall acido salicilico con un caso raro di anvelenamento nelluomo salicilato di soda, Arch. di farmacol. sper., 1905, **4**, 59.

4. Lüthje, H.: Ueber die Wirkung von Salicylpräparaten auf die Harnwege nebst einigen Bemerkungen über die Genese der Cylinder und Cylindroide, Deutsch. Arch. f. klin. Med., **74**, 163.

The object of this study is to ascertain what effect the administration of salicylate has on the morphology of the kidney and its functional efficiency as judged by certain nitrogenous constituents of the blood in animals subjected to varying conditions of dosage, mode and duration of administration of the drug. Our own work was carried out on dogs, cats and one rabbit. The dosage of salicylate used corresponded to the so-called "toxic" or full therapeutic dose used with human individuals suffering with rheumatic fever, that is, about 0.23 gm. of sodium salicylate (0.2 gm. salicylic acid) per kilogram. In some cases only one such dose was administered. In other cases, repeated doses were used with the object of producing a nephritis if possible. Usually, the salicylate (10 per cent. solution in water) was administered hypodermically. In a few instances the drug was also administered by mouth, but oral administration was not generally practiced because of loss of the drug by emesis.

As a rule, the animals were observed for two to three days before the experiment was begun, since they almost invariably showed some evidences of albuminuria. The drug was then injected and careful notes of symptoms, urinary and blood changes were made. Albumin was tested for qualitatively by the heat and acetic acid and ferrocyanid tests. The blood for nitrogen estimations was obtained directly from the heart. Nonprotein nitrogen was estimated according to the method of Folin and Denis,[5] and urea nitrogen by the urease method, using an aqueous extract of jack bean preserved with glycerin,[6] aeration and nesslerization. A microscopic examination of the urine was made regularly for the presence of leukocytes, erythrocytes and casts. In a number of animals the reaction of the blood and the reserve alkalinity before and after the administration of salicylate were estimated according to the methods of Levy,

5. Folin, O., and Denis, W.: New Methods for the Determination of Total Nonprotein Nitrogen, Urea and Ammonia in Blood, Jour. Biol. Chem., 1912, **11**, 527.

6. For this method of preparation of urease we are indebted to Dr. Cyrus H. Fiske of the Biochemical Laboratory.

Rowntree and Marriott[7] (reaction of blood) and Marriott[8] (alkali reserve).

The animals were usually killed by a few whiffs of chloroform on the expiration of a given time; necropsy was performed and the kidneys were excised. Small blocks from both organs (about 5 mm. thick) were fixed immediately in Zenker's fluid and in formaldehyd, embedded in celloidin and stained with hemalum and eosin.

The various pertinent data have been summarized in the accompanying table. For further details, the appended brief protocols of all the experiments may be consulted.

DISCUSSION

The data in the table and protocols indicate that the administration of salicylate causes the appearance of albumin, leukocytes, granular casts or cast-like bodies, and sometimes red blood corpuscles in the urine of cats, dogs and one rabbit. When these elements were present before the experiment they were increased by the administration of the drug. This is confirmatory (except appearance of erythrocytes in urine) of what was observed in human beings.

Practically all (ten) of the eleven animals, whose blood was examined, showed a variable though definite accumulation of nonprotein and urea nitrogen, an indication, therefore, of diminished renal functional efficiency so far as these tests are concerned. In Dog 17 certain of the results for urea nitrogen appear rather low. However, repeated determinations confirmed the results. There seems to be, at least in certain individuals, an increase in renal permeability for urea at an early stage in the action of salicylate as indicated by these figures. This phenomenon was previously observed, and apparently is more commonly encountered in human beings. It was partly because of this variability of the effect, that is, func-

7. Levy, R. L.: Rowntree, L. G., and Marriott, W. McK.: A Simple Method for Determining Variations in the Hydrogen-ion Concentration of the Blood, Arch. Int. Med., 1915, **16**, 389.

8. Marriott, W. McK.: A Method for the Determination of the Alkali Reserve of the Blood Plasma, Arch. Int. Med., 1916, **17**, 840.

tion seemed to be favored sometimes, other times diminished, and partly because of the small differences in the results obtained, that it was impossible to decide as to the extent of impairment in kidney function. The study of additional human cases seems to confirm what certainly appears to be the case in animals, namely, a diminution in renal functional efficiency. Just how permanent this change is cannot be definitely stated. In two of the animals there was a tendency for the urea nitrogen of the blood to return to the previous level, and in the majority of those observed over long periods of time the albuminuria gradually diminished.

So far as the reaction and the reserve alkalinity of the blood are concerned, no important changes were observed in the few animals studied.

As to morphologic changes, the data indicate that full therapeutic doses of sodium salicylate are capable of producing in animals definite lesions of the kidney, easily demonstrable by microscopic examination. In the case of Animal 17, oral administration of the drug produced a renal lesion quite as severe, both functionally and morphologically, as followed subcutaneous administration.

Considering the series as a whole, the administration of the drug was followed by histologic lesions varying from slight cloudy swelling of the tubular epithelium to severe cloudy swelling associated in a few cases with necrosis and desquamation, and in nine instances with distinct glomerular lesions. The glomerular lesions were never very severe and consisted principally of swelling and multiplication of capillary endothelium. In these cases the severe involvement of the tubules seemed to justify emphasizing that phase in the diagnosis rather than making a diagnosis of acute glomerular nephritis. The finding of albuminous granular precipitate in the subcapsular space, in the opinion of the writers, does not justify a definite diagnosis of glomerular lesion; accordingly several cases diagnosed as cloudy swelling show in addition this minor change.

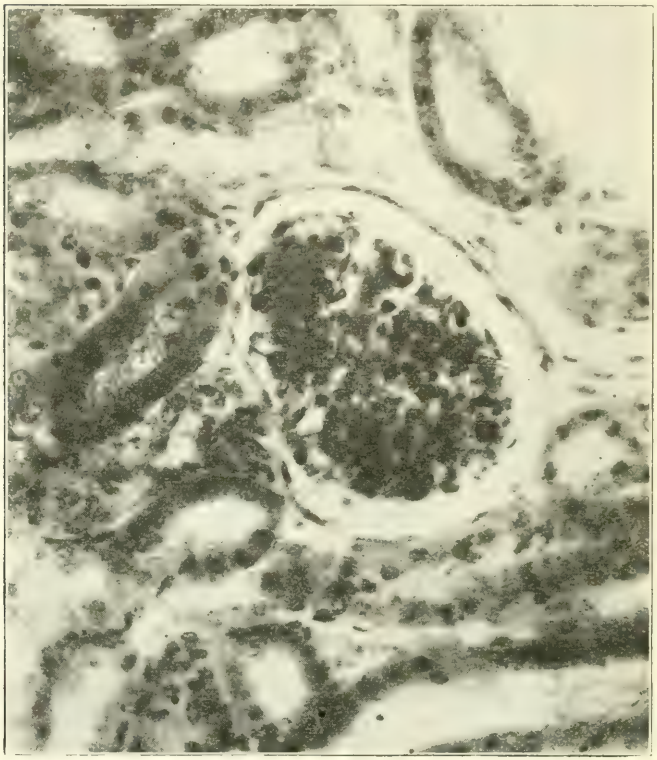
The proximal convoluted tubule was apparently the first part attacked followed very rapidly by lesion of the distal convoluted tubule, then of the ascending limb of the loop of Henle. As a rule, it appeared that these divisions were involved before lesion of the glomerulus occurred. In cases in which all these features appeared, the lesion of the proximal convoluted tubule was the most severe change. No stress is laid on fatty changes, because, independently of experimental procedures, such changes are practically constant in the cat and frequent in both the dog and rabbit. The diagnosis of necrosis of tubular epithelium in these kidneys rests in part on finding distinct nuclear pyknosis, and in part on finding desquamation of entire cells and groups of cells. The accompanying figure illustrates a renal lesion.

Although Vinci found differences in the susceptibility of dogs, guinea-pigs and rabbits, the experiments reported in this paper show no material differences in susceptibility of dogs and cats. He found essentially the same type and distribution of tubular lesion as we have described, but in contrast to the definite intracapillary glomerulitis seen in some of our animals, he describes only marked congestion of the tuft. In his series and ours there was found cloudy swelling of glomerular epithelium and albuminous material in the subcapsular space. Vinci's failure to find the more marked glomerular lesion is probably due to the fact that most of his animals died or were killed after only seven or eight hours.

It is not possible in this series definitely to correlate the morphologic and functional changes. It will be noted as an indication of this difficulty that the most marked accumulation of urea nitrogen occurred in connection with a kidney that histologically showed no change in the glomeruli except cloudy swelling of the lining epithelium and albuminous precipitate in the subcapsular space (Experiment 4).

An interesting fact, which may or may not have a bearing on the problem of albuminuria and nephritis, is a diminution in the total excretion of salicylate in

the urine of Dog 18 as compared with the urinary excretion of Dog 19. The kidney of Dog 18 shows a definite, acute tubular nephritis which, in part, at least, may have been present before the animal received sali-



Kidney of Dog 12 showing slight involvement of the glomerular tuft, which, although not enlarged, is richly cellular and devoid of blood. The tubular epithelium shows cloudy swelling and the lumina contain granular albuminous precipitate.

cylate. Dog 19 was in good condition and the kidneys show cloudy swelling without glomerular change. Relatively, the accumulation of urea nitrogen in the blood of Dog 18 is also greater than that of Dog 19.

Whatever the explanation may be, it is nevertheless interesting to note that the animal showing the more profound renal functional and morphologic changes excreted less salicylate than the animal with the less pronounced changes. This coincides with certain observations reported by Hanzlik, Scott and Thoburn[9] on the excretion of salicylate in human beings with different clinical conditions as compared with normal individuals.

CONCLUSIONS

1. The administration of salicylate in doses corresponding to full therapeutic doses for human beings per kilogram of body weight, causes the appearance of albumin, leukocytes, casts or cast-like bodies, and sometimes red blood corpuscles, in the urine of animals (cats, dogs and one rabbit).

2. A preexisting albuminuria is aggravated by the administration of salicylate.

3. The albuminuria is of direct renal origin.

4. So far as the nonprotein and urea nitrogen of the blood are concerned, there is a diminution in renal functional efficiency.

5. Morphologically, a lesion of the kidney appears, varying in severity from simple cloudy swelling of the epithelium of the proximal convoluted tubule to extensive cloudy swelling of all the cortical parts of the tubules, associated with an acute intracapillary glomerulitis, the latter process being denominated as an acute tubular nephritis.

PROTOCOLS

Experiment 1.—Dog; 3.1 kg. Urine before salicylate was albumin-free; received 0.3 gm. sodium salicylate per kilogram by mouth; one hour later there was marked albuminuria. On the following day there was still marked albuminuria; received two doses of 0.3 gm. each per kilogram, but vomited almost immediately after each dose; 4 gm. of salicylate were

9. Hanzlik, P. J.; Scott, R. W., and Thoburn, T. W.: The Salicylates. V. Secretion of Salicyl in the Urines of Rheumatic and Non-Rheumatic Individuals, Jour. Pharmacol. and Exper. Therap., 1917, **9**, 247.

then injected in three divided doses hypodermically within thirty minutes; animal was killed one and one-quarter hours after the last dose. Albuminuria was severe; red and white cells were present in the urine.

Histologically the glomeruli show slight congestion, but otherwise normal tufts; occasional glomeruli show albuminous precipitate in the subcapsular space. Epithelium of the proximal convoluted tubules shows moderate cloudy swelling; the lumina of these tubules are normal. There are no casts.

Diagnosis: Cloudy swelling of kidney.

Experiment 2.—Dog; 5.4 kg. Urine before salicylate contained traces of albumin, which was markedly increased, together with the appearance of white and red cells after the subcutaneous injection of 0.9 gm. sodium salicylate. Two days later the animal received 3.3 gm. salicylate in two divided doses. The animal died during the night; urine from bladder gave strong albumin tests and showed the presence of white and red corpuscles.

Histologic picture somewhat obscured by postmortem degeneration. Glomerular tufts normal save for slight congestion; the subcapsular spaces contain much granular precipitate and also a moderate number of desquamated capsular epithelial cells; this latter condition, however, is probably the result of postmortem changes. The epithelium of the convoluted tubules shows marked granularity and the lumina contain many albuminous granules. There are no casts. Other tubules show less marked granular change. Around some of the large blood vessels there is an old connective tissue growth.

Diagnosis: Postmortem degeneration and probably cloudy swelling of kidney.

Experiment 3.—Dog; 5.6 kg. For four days previous to injection of salicylate, the urine showed a trace of albumin. On day of experiment the urine was albumin-free. Received single injection of 0.05 gm. salicylate per kilogram hypodermically. Seven hours later the urine gave strong albumin tests and showed the presence of white and red corpuscles; dog found dead in cage next morning; 5 c.c. of urine obtained from the bladder; this gave strong albumin tests, and showed numerous white corpuscles and granular cast-like bodies.

Postmortem degeneration has advanced to such a degree as to render histologic description useless.

Experiment 4.—Cat; 3.2 kg. Urine before salicylate showed questionable trace of albumin for two days; 1.05 gm.

sodium salicylate was injected in three divided doses in the course of forty-five hours; 700 c.c. water were administered at different times and about 300 c.c. urine were voided during this interval. The animal was killed. The urine at all times gave marked albumin tests and showed many white, and a few red corpuscles.

Histologically the glomeruli show practically normal vascular tufts; the subcapsular space in many instances shows a moderate amount of albuminous precipitate; a small number of glomeruli show swelling of the capsular epithelium. The epithelium of the proximal convoluted tubules show well-marked granularity of the protoplasm, in some instances hyaline droplet formation, and infrequently many desquamated epithelial cells. The tubules otherwise are normal; there are no casts.

Diagnosis: Cloudy swelling of kidney. The change might be called by some authors an acute tubular nephritis, but without more marked tubular change, and especially in the absence of notable change in the glomerular tuft, such a diagnosis seems hardly justifiable.

Experiment 5.—Cat; 3 kg. Trace of albumin present in urine for a week before salicylate; 0.9 gm. sodium salicylate injected in two successive days; total injected 1.8 gm. Following second injection, marked albuminuria, few granular casts; many leukocytes, few erythrocytes; animal was killed in a dying condition on the second day.

Histologically the interstitial tissue is normal. The glomeruli show normal capsules and no exudate in the subcapsular space, but the vascular tufts are large, fill the capsular spaces and are almost devoid of blood. The epithelium of the cortical tubules contains much fat, but marked granularity of the protoplasm can easily be made out, and the lumina of many of the tubules contain fine and coarsely granular albuminous precipitate.

Diagnosis: Acute tubular nephritis.

Experiment 6.—Cat; 2.1 kg. Trace of albumin in urine before salicylate; 0.6 gm. sodium salicylate injected in two doses within twenty-six hours; total injected 1.2 gm. Marked albuminuria together with granular casts and leukocytes. Animal markedly depressed; killed.

Histologically, the interstitial tissue and the glomeruli are in all respects normal. The epithelium of proximal and distal convoluted tubules, as well as of the ascending loop of Henle, show various stages of cloudy swelling, from swelling of the epithelium with occlusion of the lumen to narrowing of the epithelial lining, with the presence of a considerable

amount of granular precipitate in the lumina. There are a few hyaline casts and a few minute interstitial hemorrhages.

Diagnosis: Slight cloudy swelling; almost a normal kidney.

Experiment 7.—Rabbit; 1.2 kg. Urine free from albumin before salicylate; 2.2 gm. sodium salicylate injected in four doses on three successive days. Following each injection, mild albuminuria, together with occasional granular and hyaline casts. Throughout the experiment the animal appeared normal; killed.

Histologically, the interstitial tissue shows numerous small areas of overgrowth with associated atrophy of tubules and glomeruli. In the large areas between these foci the interstitial tissue is apparently normal. The glomerular capsules and subcapsular spaces show nothing pathologic. The vascular tufts are large, bloodless and apparently the seat of an acute glomerulitis. In somewhat irregularly distributed areas the convoluted tubules show marked granularity, vagueness of outline, fragmentation and desquamation, with slight pyknosis of a few of the nuclei. The tubular lumina contain granular albuminous precipitate and a few hyaline casts.

Diagnosis: Acute tubular nephritis superimposed on chronic interstitial nephritis.

Experiment 8.—Cat; 1.9 kg. Slight albuminuria present before salicylate. A single injection of 0.6 gm. sodium salicylate was given. Animal found dead in cage next morning; bladder urine contained large quantity of albumin and casts.

Histologically, the interstitial tissue and the glomeruli are normal, except that a very few of the latter show large, bloodless, richly cellular, vascular tufts. The epithelium of the cortical tubules is more granular than normal and occupies a decreased space in the tubule. The correspondingly enlarged lumina are filled with finely and coarsely granular albuminous precipitate. Several arched collecting tubules contain hyaline casts.

Diagnosis: Acute tubular nephritis.

Experiment 9.—Cat; 1.3 kg. Trace of albumin in urine before salicylate; 0.85 gm. sodium salicylate injected in two doses on two successive days. Animal died in one and one-half hours after second injection; kidneys excised immediately after death; bladder urine gave strong tests for albumin; granular casts present.

Histologically, the interstitial tissue is normal, as are also the glomerular capsules and subcapsular spaces. The glomerular tufts are large, devoid of blood and richly cellular.

The epithelium throughout the cortex shows cloudy swelling, but in the proximal and distal convoluted tubules the granulation is very marked; there is fragmentation and desquamation of cells, the tubular lumina are filled with albuminous precipitate and an occasional narrow hyaline cast can be seen.

Diagnosis: Acute tubular nephritis.

EXPERIMENT 10.—Dog; 6.5 kg. Urine free from albumin and casts before salicylate; 6.05 gm. sodium salicylate injected in four divided doses on four successive days. Within one to two hours after each of the first two injections the dog was depressed, with increased respiration. On the day of the third injection the animal appeared normal and in good condition, but following the third and fourth injections of the drug the animal became progressively more depressed and weak and died sometime during the following night and day (over Sunday). Albuminuria, together with leukocytes and considerable epithelium, appeared following the first injection and progressively increased until death (at times red corpuscles were present). Kidneys not sectioned because of postmortem changes.

EXPERIMENT 11.—Dog; 6.3 kg. Mild albuminuria, together with leukocytes present before salicylate. Sodium salicylate was injected in three divided doses on three successive days as follows: 0.3 gm. daily for the first two days, and 1.89 gm. on the third day; total injected, 2.10 gm. Dog vomited and showed considerable depression after each injection. Albuminuria, together with casts, leukocytes and epithelium, progressively increased until the animal was killed on the third day. Blood was obtained from the heart daily for urea nitrogen determination; pericardium and myocardium appeared normal.

Histologically, the interstitial tissue and glomeruli are normal. The cortical tubules show a narrowed epithelial lining, the cells of which show increased granularity and fragmented, ragged edges; the tubular lumina are well filled with granular albuminous precipitate.

Diagnosis: Cloudy swelling.

EXPERIMENT 12.—Dog; 5.5 kg. Faint trace of albumin in urine before salicylate; 3.5 gm. sodium salicylate injected in two doses on successive days. Considerable depression and vomiting followed second injection. Marked albuminuria, together with granular casts, leukocytes, some erythrocytes and clumps of epithelium present in urine. Animal killed.

TABLE SHOWING EFFECT OF SALICYLATE IN KIDNEY[1]

Number and Animal	Body Weight, Kg.	Total Quantity of Sod. Salicylate[2] Administered, Gm.	No. of Toxic Doses	Mode of Administration	Albuminuria		N.P.N. and Urea-N in Blood (Mg. in 100 C.c.)		Reaction of Blood (pH) and Alkali Reserve (RpH)		Pathologic Diagnosis	Experiment Terminated End of
					B. S.	A. T.	B. S.	A. T.	B. S.	A. T.		
Dog 1	3.1	4.6	6	Hypo. + per os	+st	+st	.....	.....	.....	.....	Cloudy swelling	2 days
Dog 2	5.4	4.2	3	Hypo.	+tr	+st	.....	.....	.....	.....	Cloudy swelling (?)	3 days
Dog 3	5.6	0.05	1	Hypo.	–	+	N.P.N. 52.0	62.0	.....	.....	.....	2 days
Cat 4	3.2	1.05	3	Hypo.	+?	+	N.P.N. 22.4	130.4	.....	.....	Cloudy swelling	45 hours
Cat 5	3.0	1.8	2	Hypo.	+tr	+	.....	.....	.....	.....	Acute tubular nephritis	3 days
Cat 6	2.1	1.2	2	Hypo.	+sl	+st	.....	.....	.....	.....	Very slight cloudy swelling	26 hours
Rabbit 7	1.2	2.2	4	Hypo.	+sl	+st casts	.....	.....	.....	.....	Acute tubular nephritis superimposed on chronic interstitial nephritis	3 days
Cat 8	1.9	0.6	1	Hypo.	+sl	+st	.....	.....	.....	.....	Acute tubular nephritis	20 hours (?) (Died during night)
Cat 9	1.3	0.85	2	Hypo.	+sl	+st	.....	.....	pH 7.4	pH 7.4	Acute tubular nephritis	20 hours
Dog 10	6.5	6.05	4	Hypo.	—	+	N.P.N. 20.0	(1) 40.2 (2) 49.0 (2) 66.7 (4) 80.0	.....	.....	(No sections)	4 days
Dog 11	6.3	2.19	3	Hypo.	+sl	+	Urea N 24.5	(1) 24.2 (2) 18.0 (3) 40.3	pH 7.6	pH 7.7 7.6 7.6	Cloudy swelling	5 days

Dog 12	5.5	3.5	2	Hypo.	+ vsl	+	Urea N 6.03	(1) 6.2 (2) 8.73 (3) 18.2	pH 7.7	pH 8.0		Cloudy swelling	5 days
Cat 13	2.1	0.94	1	Hypo.	+	+	Urea N 20.4	20.3	pH 7.6 RpH 8.2	pH 7.6 RpH 8.2		Acute tubular nephritis	26 hours
Cat 14	0.5	0.15	1	Hypo.	No urine	No urine	Urea N 12.8	12.7	.....	.....		Acute tubular nephritis	6½ hours
Cat 15	0.5	0.15	1	Hypo.	No urine	No urine	Urea N 16.1	.....	.....	.....		Slight cloudy swelling	6½ hours
Cat 16	2.0	0.6	1	Hypo.	+ sl	+ casts	Urea N 20.1	25.8	.....	.....		Acute tubular nephritis	9 hours
Dog 17	7.5	3.0	2	Per os	+	+	Urea N 8.56	(1) 4.1 (2) 3.3 (3) 6.2 (4) 12.5 (5) 12.8 (6) 18.0 (7) 18.2 (8) 19.3 (9) 14.0	pH 7.8 RpH 8.4	pH 7.7 RpH 8.2 7.8 8.2 7.8 8.2 7.5 8.5 7.7 8.4 7.6 8.2 7.6 8.4 7.6 8.6 7.6 8.4		Acute tubular nephritis	9 days
Dog 18[3]	4.0	1.2	1	Hypo.	+	+	Urea N 7.1	(1) 12.8 (2) 12.8 (3) 16.0 **(4) 18.6**	.....	.....		Acute tubular nephritis	4 days
Dog 19[4]	5.8	1.3	1	Hypo.	—	+	Urea N 11.0	(1) 14.0 (2) 14.1 (3) 9.5 (4) 10.0	.....	.....		Marked cloudy swelling	4 days

1. The abbreviations in the table have meanings as follows: B. S. = before salicylate; A. T. = at "toxicity"; or when the effects of the salicylate were evident as indicated by symptoms of nausea, vomiting, loss of equilibrium, etc.; N.P.N. = nonprotein nitrogen; pH = hydrogen-ion concentration; RpH = reserve alkalinity; st = strong; sl = slight; + = present; — = absent; figures in parentheses refer to days.

2. Sodium salicylate contains 86 per cent. salicylic acid.

3. Total quantity of salicyl recovered in urine = 26.6 per cent. The distillation colorimetric method was used as described by Thoburn and Hanzlik: Jour. Biol. Chem., 1915, 23, 163.

4. Total quantity of salicyl recovered in urine = 55.4 per cent.

Histologically, the interstitial tissue is normal, as also are the glomeruli, except for a slight deposit of granular albuminous material in the subcapsular space. The epithelium of the cortical tubules is more granular than normal and the tubular lumina, increased in caliber, contain much albuminous granular precipitate.

Diagnosis: Cloudy swelling.

Experiment 13.—Cat; 2.1 kg. Albuminuria present before salicylate; 0.94 gm. sodium salicylate injected as follows: 0.62 gm. on the first day, 0.32 gm. on the second day. Convulsions of medullary type occurred about eight hours after the first injection, and cat died in about two hours after the second injection. Albumin in urine present after each injection, though not marked.

Histologically, the interstitial tissue is normal. The glomerular tufts are large, richly cellular and bloodless. The convoluted tubules show a moderate amount of fat. The cortical tubules as a whole show marked granularity of cell protoplasm, in several places, desquamation. The tubular lumina contain albuminous granular material and several of the distal convoluted tubules show an accumulation of hyaline material in the lumina, not as yet fused and separated to form casts.

Diagnosis: Acute tubular nephritis.

Experiment 14.—Kitten; 0.5 kg. No urine obtainable at any time. Single dose of 0.15 gm. sodium salicylate injected. Animal remained in good condition; killed at the end of sixteen and a half hours after injection.

The histologic changes in this kidney are practically the same as those seen in Animal 13.

Diagnosis: Acute tubular nephritis.

Experiment 15.—Kitten; 0.5 kg. No urine obtainable at any time. Single dose of 0.15 sodium salicylate injected. Considerable depression at the end of six hours after injection; died while attempting to secure blood from heart.

Histologically, the interstitial tissue is normal. The glomeruli are large, contain a moderate amount of blood and there is a small amount of granular albuminous precipitate in the subcapsular spaces.

The cortical tubular epithelium shows slightly increased granularity and the tubular lumina contain a small amount of granular albuminous material.

Diagnosis: Slight cloudy swelling.

Experiment 16.—Cat; 2 kg. Slight albuminuria, together with leukocytes before salicylate. Single dose of 0.6 gm. sodium salicylate injected. Albuminuria considerably increased; leukocytes numerous; some erythrocytes and cast-like bodies. Animal killed at end of nine hours after injection.

Histologically, the interstitial tissue is normal. The vascular tufts of the glomeruli are large, contain an occasional erythrocyte, but are more richly cellular than normal and frequently show filling of capillary loops by endothelium. The subcapsular spaces contain albuminous granular precipitate. The cortical tubules show well marked cloudy swelling, most marked in the proximal convoluted tubules, where the cells are large and extremely granular, and the tubular lumina are filled with much albuminous granular precipitate.

Diagnosis: Acute tubular nephritis.

Experiment 17.—Dog; 7.5 kg. Albuminuria present, but no casts before salicylate. On the first day 0.25 gm. sodium salicylate was administered into the stomach every hour until six doses were given; total salicylate administered, 1.5 gm. Urine was expressed from the bladder at intervals, but contained no more albumin than before salicylate; no casts; occasional leukocytes and erythrocytes present; no vomiting; no depression. On the second day 1.5 gm. salicylate were administered in the same way as on the first day; total salicylate administered in experiment, 3 gm. Vomiting occurred after the last dose was administered; the animal showed some depression and incoordination; more vomiting occurred during the following night. The animal was observed continuously for seven days after the last injection; total observation period, nine days. Albuminuria increased gradually up to the fourth day after injection of salicylate, then gradually diminished until the last day, when only a fairly positive trace of albumin was present; no casts at any time. The animal gained weight slightly (160 gm.) and remained in good condition throughout the experiment. Blood from the heart was obtained daily for urea nitrogen and reaction estimations; killed on the ninth day.

Histologically, the interstitial tissue is normal. The vascular tufts of the glomeruli are large, bloodless and richly cellular. The subcapsular spaces contain granular albuminous precipitate. The cortical tubules show cloudy swelling, most markedly in the proximal convoluted tubules, where there is

marked granularity of the epithelium as well as fragmentation and desquamation. The tubular lumina show much granular albuminous material and a few epithelial and coarsely granular casts.

Diagnosis: Acute tubular nephritis.

Experiment 18.—Dog; 4 kg. Albumin present (more than a trace) and some leukocytes before salicylate. A single dose of 1.2 gm. sodium salicylate (actually 1.32 gm. salicylic acid) was injected hypodermically. Animal observed for next four days. Dog originally was not very vigorous; no depression from salicylate, but progressively became morose, emaciated and emitted a foul odor from the oral cavity, which showed signs of infection; site of salicylate injection was edematous and necrotic. Albuminuria, together with leukocytes, present throughout experiment, and remained about the same after the salicylate. Blood was secured from the heart daily for urea nitrogen estimation; killed on fourth day. Pericardium and myocardium appeared normal. Left kidney showed about ten large, irregular white patches diffusely scattered on the surface; in some places these appeared to be raised, resembling miliary tubercles. The right kidney showed three such patches. Other viscera appeared normal. The total quantity of salicylate recovered from the cage contents (urine, etc.) was 26.6 per cent., expressed as salicylic acid.

Histologically, the interstitial tissue is normal. The glomeruli show large, bloodless, richly cellular vascular tufts and a small amount of finely granular albuminous precipitate in the subcapsular space. The cortical tubular epithelium shows cloudy swelling, most markedly in the proximal convoluted tubules, where there is marked granularity, fragmentation and desquamation, the tubular lumina containing granular albuminous material but no casts.

Diagnosis: Acute tubular nephritis.

Experiment 19.—Dog; 5.8 kg. Urine practically free from albumin before salicylate. Single dose of 1.3 gm. sodium salicylate (actually 1.235 gm. salicylic acid). Animal became nauseated, but no vomiting occurred; for two days following the injection, the albuminuria increased, then gradually disappeared, only a trace being present on the last day of the experiment. Blood was secured daily from the heart for urea nitrogen estimation; the animal remained in good condition; killed on the fourth day; all viscera appeared normal. The total quantity of salicylate recovered from the cage contents (urine, etc.) was 55.4 per cent., expressed as salicylic acid.

Histologically, the interstitial tissue is normal. The glomeruli are normal, except that the subcapsular spaces frequently contain granular albuminous precipitate. The cortical tubular epithelium, as in Dog 18, shows cloudy swelling, most markedly in the proximal convoluted tubules where there is marked granularity, fragmentation and desquamation, the tubular lumina containing granular albuminous material but no casts.

Diagnosis: Marked cloudy swelling.

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THE SALICYLATES

VII. FURTHER OBSERVATIONS ON ALBUMINURIA AND RENAL FUNCTIONAL CHANGES FOLLOWING THE ADMINISTRATION OF FULL THERAPEUTIC DOSES OF SALICYLATE *

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(*From Archives of Internal Medicine, June, 1917*)

In a previous communication[1] it was shown that the administration of salicylate in full therapeutic doses invariably causes the appearance of albumin, together with leukocytes and casts or cast-like bodies, in the urines of rheumatic and nonrheumatic individuals. The observations on renal function were inconclusive because of the variations and small differences in both the phenolsulphonephthalein excretion and nonprotein nitrogen of the blood before and after the administration of the drug. It was definitely shown, however, that the albuminuria is not of febrile, but of direct renal origin. The albuminuria, in some cases, is so severe that the presence of a nephritis is suggested. This was found to be the case in animals[2] (cats, dogs and a rabbit) receiving quantities of salicylate per kilogram of body weight corresponding to the full therapeutic dose in human beings, in single and repeated doses, by mouth and subcutaneously. The

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1. Scott, R. W., and Hanzlik, P. J.: Jour. Am. Med. Assn., 1916, **67**, 1838.

2. Hanzlik, P. J., and Karsner, H. T.: Arch. Int. Med., 1917, **19**, 1016.

pathologic changes in the kidney were interpreted as an acute tubular nephritis. An accumulation of nonprotein and urea nitrogen in the bloods of a majority of the animals was also demonstrable. In other words, salicylate, in these animals, caused a diminution in renal functional efficiency, so far as these tests are concerned.

These findings encouraged us to extend our former observations, and arrive, if possible, at some final decision concerning the effects of salicylate on renal function in human beings. If it could be definitely shown that the drug injures the kidney, it should engender caution in its careless or promiscuous use as a remedy, particularly as to repeated administrations.

It is the object of this paper to summarize the results of all of our observations on the effects on human renal functional efficiency. Observations on twelve additional individuals have been added to the sixteen previously reported, making a total, therefore, of twenty-eight different individuals, and thirty-two observations. A definite quantity (20 c.c.) of salicyl (usually of about 10 per cent. strength) in the form of sodium salicylate was administered, together with 80 c.c. of water, every hour until the subject complained of the well known symptoms of "toxicity." Then the administration was stopped, but the water was continued, usually 200 c.c. every two hours, until the excretion of salicyl in the urine ceased. That is, the fluid intake was maintained constant throughout as nearly as possible. Urine was collected every ten hours throughout the experiment and examined qualitatively (by the heat and acetic acid and ferrocyanid tests), and quantitatively (by the gravimetric method of Folin and Denis) for the presence of albumin, and microscopically for leukocytes, casts, etc., before and after the administration of the drug. Other tests of renal functional efficiency (before and after the administration of salicyl) were made as follows: The total nonprotein nitrogen of the blood was estimated according to the method of Folin and Denis,[3] using titration

3. Folin and Denis: Jour. Biol. Chem., 1912, **11**, 527.

TABLE 1.—CLINICAL DATA*

Number and Patient	Diagnosis	Condition of Temperature	Fluid Intake	Toxic Dose of Salicyl† Gm.	Diaphoresis	Urinary Changes: Albumin, Before Salicyl	Urinary Changes: Albumin, During Salicyl Medication	Urinary Changes: White Blood Corpuscles, Before Salicyl	Urinary Changes: White Blood Corpuscles, During Salicyl Medication	Urinary Changes: Granular Casts or Castlike Bodies, Before Salicyl	Urinary Changes: Granular Casts or Castlike Bodies, During Salicyl Medication	Administered Sodium Bicarbonate Together With Salicyl, Gm.
1 (P. F.)‡	Rheumatic fever	Febrile	256 c.c. every hour for 12 hrs.; 256 c.c. every 2 hrs. thereafter	13.41	Moderate	—	+					
2 (J. M.)	Rheumatic fever	Febrile	170 c.c. every hour for 8 hrs.; 300 c.c. every 2 hrs. thereafter	12.81	Profuse	—	+	—	+	—	+	
3 (W. C.)	Rheumatic fever	Febrile	100 c.c. every hour for 9 hrs.; 200 c.c. every 2 hrs. thereafter	15.48	Profuse	+tr.	+inc.	+oc.	+inc.	+oc.	+inc.	
4 (J. H.)‡	Rheumatic fever	Febrile	220 c.c. every hour for 8 hrs.; 400 c.c. every 2 hrs. thereafter	14.45	Profuse	+	+inc.	—	—	—	+	
5 (J. L.)‡	Rheumatic fever	Febrile	200 c.c. every hour for 8 hrs.; 400 c.c. every 2 hrs. thereafter	14.45	Very profuse	+tr.	+inc.	+oc.	inc.	—	oc.	
6 (F. D)	Appendicitis T. B. kidney (?)	Afebrile	180 c.c. every hour for 7 hrs.; 300 c.c. every 2 hrs. thereafter	12.04	Mild	+tr.	+inc.	+oc.	+inc.	—	—	
7 (L. E.)	Tabes	Afebrile	150 c.c. every hour for 8 hrs.; 300 c.c. every 2 hrs. thereafter	13.76	Marked	—tr.	+inc.	+oc.	+inc.	—	+	
8 (H. M.)	Normal	Afebrile	150 c.c. every hour for 9 hrs.; 400 c.c. every 2 hrs. thereafter	15.48	Mild	—	+	+oc.	inc.			
9 (A. P.)	Normal	Afebrile	100 c.c. every hour for 12 hrs.; 200 c.c. every 2 hrs. thereafter	14.96	Very mild	—	+	—	+	—	+	
10 (J. B.)	Normal	Afebrile	100 c.c. every hour for 11 hrs.; 200 c.c. every 2 hrs. thereafter	19.30	.....	+tr.	—inc.	—	+	—	—	
11 (S. V.)	Rheumatic fever	Febrile	100 c.c. every hour for 11 hrs.; 200 c.c. every 2 hrs. thereafter	18.33	Moderate	+tr.	+inc.	+oc.	+inc.	+oc.	+inc.	
12 (W. B.)	Rheumatic fever Old T. B. lesion of apex	Febrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	8.75	Very profuse	+tr.	—inc.	—		—		
13 (D. T.)‡	Normal	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	13.3	Mild	+tr.	+inc.	—		—	—	14.0
14 (J. T.)‡	Normal	Afebrile	100 c.c. every hour for 6 hrs.	10.5	Mild	—tr.	+inc.	+oc.	inc.	—	—	
15 (M. Z.)‡	Normal	Febrile	100 c.c. every hour for 6 hrs.	10.5	Marked	+st.	+inc.	+	+inc.	+	+	
16a (M. C.)‡	Lessened renal functional efficiency; hysteria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	12.3	Mild	+tr.	+inc.	+oc.	+inc.	+oc.	+inc.	

16b(M. C.)‡	Lessened renal functional efficiency; hysteria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	11.4	Mild	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	12.0
16c (M. C.)‡	Lessened renal functional efficiency; hysteria	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	10.5	Mild	+	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	
16d(M. C.)‡	Lessened renal functional efficiency; hysteria	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	9.36	Mild	+ tr.	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	
17 (P. K.)	Chronic alcoholism	§	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.3	Mild	+	+ inc.	+ oc.	+ inc.	+ oc.	+ inc.	14.0
18 (F. S.)	Chronic alcoholism	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Profuse	+	+ inc.	+	+ inc.	+	+ inc.	
19 (Y. V.)	Tuberculosis	Very febrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Moderate	+	+ inc.	+	+ inc.	+	+ inc.	
20 (G. W.)	Chronic morphinism	Afebrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.8	Mild	tr.	inc.	+	+ inc.	+	+	
21a(J. V.)	Rheumatic fever	Febrile	100 c.c. every hour for 6 hrs.; 200 c.c. every 2 hrs. thereafter	10.2	Profuse	+	+ inc.	+	+ inc.	+	+	
21b(J. V.)	Normal	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.5	Mild	—	inc.	—	+	—	—	
22 (E. L.)	Surg. (?)¶	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Mild	tr.	inc.	—	+	—	+	
23 (E.)	Chronic rheumatism	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.5	Marked	+	+ inc.	—	+ inc.	+	+ inc.	
24 (J. H.)	Endocarditis; cardiac decompensation	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	8.1	Mild	+	inc.	+	+ inc.	+	+ inc.	
25 (H. V.)	Normal; photophobia	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Mild	—		—	+	—	—	
26 (R. H.)	Normal; ulcer of eye	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.6	Marked	+	inc.	—	+ inc.	+ oc.	+	
27 (J. M.)	Normal; varicose ulcer	Afebrile	100 c.c. every hour for 7 hrs.; 200 c.c. every 2 hrs. thereafter	12.6	Mild	+	inc.	+	+	oc.	+	14.0
28 (T. S.)	Normal	Afebrile	100 c.c. every hour for 5 hrs.; 200 c.c. every 2 hrs. thereafter	9.0	Very mild	—		—	+	—	+ oc.	9.0

* In this table the various signs and abbreviations have meanings as follows: plus (+) sign, present; minus (—) sign, absent; tr., trace; inc., increased; oc., occasional; st., strong.

† Salicyl was administered in form of sodium salicylate, expressed as salicylic acid.

‡ The albuminuria in this individual was not studied quantitatively. In such cases, the urines were either utilized for other quantitative studies, or they had stood too long to give trustworthy results for albumin.

§ Partially febrile, temperature reaching a maximum of 99.8 F.

¶ A surgical condition, possibly renal; diagnosis incomplete.

and distillation instead of the colorimetric procedure. The urea nitrogen content of the blood was estimated by the urease, aeration and colorimetric procedure.[4] The percentage excretion of phenolsulphonephthalein in two hours was studied in the usual way. The various clinical data pertaining to all the subjects are summarized in Table 1.

TABLE 2.—EXCRETION OF ALBUMIN IN URINE OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALCYLATE

No.	Grams of Albumin Excreted in Each Ten-Hour Period									
	B. S.*	1	2	3	4	5	6	7	8	9
2	0.0	0.43	0.14	0.09	0.05	0.11	0.0	0.0	0.0	0.0
3	0.0	0.12	0.48	0.41	0.32	0.31	0.18	0.0	0.0	0.0
6	0.0	0.13	0.07	0.16	0.42	0.10	0.37	0.09	0.03	0.0
7	0.0	0.16	0.06	0.03	0.02	0.01	0.0	0.0	0.0	0.0
8	0.0	0.32	0.03	0.27	0.14	0.02	0.49	0.0	0.0	0.0
9	0.0	0.0	0.0	0.05	0.08	0.03	0.01	0.01	0.0	0.0
10	0.21	0.69	0.43	0.66	0.70	0.72	0.60	0.22	0.29	0.11
11	0.10	0.13	0.18	0.11	0.25	0.18	0.16	0.04	0.06	0.04
12	0.09	0.29	0.10	0.04	0.10	0.04	0.07	0.18	0.10	0.10
16b†	0.003	0.03	0.21	0.09	0.07	0.03	0.01	0.02	0.08	0.01
16c	0.05	0.01	0.10	0.08	0.05	0.15	0.08	0.02	0.06	0.005
16d	0.002	0.01	0.10	0.02	0.12	0.06	....	0.07	0.0	0.0
17†	0.04	0.20	0.05	0.24	0.06	0.10	0.06	0.02	0.03	0.14
18	0.11	0.16	0.13	0.05	0.08	0.03	0.09	0.05	0.03	0.04
19	0.06	0.19	0.27	0.47	0.42	0.25	0.31	0.38	0.003	0.13
20	0.01	0.38	0.28	0.02	0.006	0.02	0.07	0.05	0.05	0.02
21a	0.22	0.32	0.55	0.48	1.17	0.16	0.01	....	....	0.13
22	0.06	0.05	0.09	0.04	0.05	0.04	0.02	0.01	0.03	0.0
21b	0.0	0.04	0.13	0.06	0.02	0.0	0.0	0.0	0.0	0.0
23	0.02	0.07	0.08	0.03	0.01	0.09	0.11	0.0	0.26	0.08
24	0.0	0.06	0.31	0.22	0.23	0.24	0.05	0.08	....	0.04
25	0.0	0.06	0.09	0.12	0.14	0.08	0.06	0.02	0.0	0.0
26	0.05	0.28	0.12	0.12	0.46	1.98	0.58	0.21	0.0	0.0
27†	0.04	0.18	0.38	0.35	0.06	0.02	0.09	0.05	0.0	0.0
28†	0.0	0.09	0.03	0.04	0.02	0.07	0.05	0.02	0.0	0.0
Median	0.01	0.13	0.12	0.10	0.08	0.09	0.07	0.02	0.02	0.03

* B. S. means "before salicyl."
† Received bicarbonate together with salicylate.

1. *Salicyl Causes Albuminuria and Aggravates a Preexisting Albuminuria.*—In all the individuals of the present series, the administration of salicylate was followed by the presence of albumin, leukocytes and granular casts or cast-like bodies in their urines. This is, therefore, confirmative of our former observations. It is further confirmed that the albuminuria is not of

4. We wish to express our thanks to Dr. Cyrus H. Fiske of the Biochemical Laboratory for suggesting the use of a glycerin extract of the jack bean for the urease.

febrile, but of direct renal origin, for it occurs in febrile, afebrile, rheumatic, nonrheumatic and normal persons.

Many of the individuals showed some degree of albuminuria before the drug was administered. It was, therefore, necessary to study the excretion quantitatively in order to ascertain definitely if this was increased by the salicylate. This was done by the gravimetric method of Folin and Denis.[5] The results

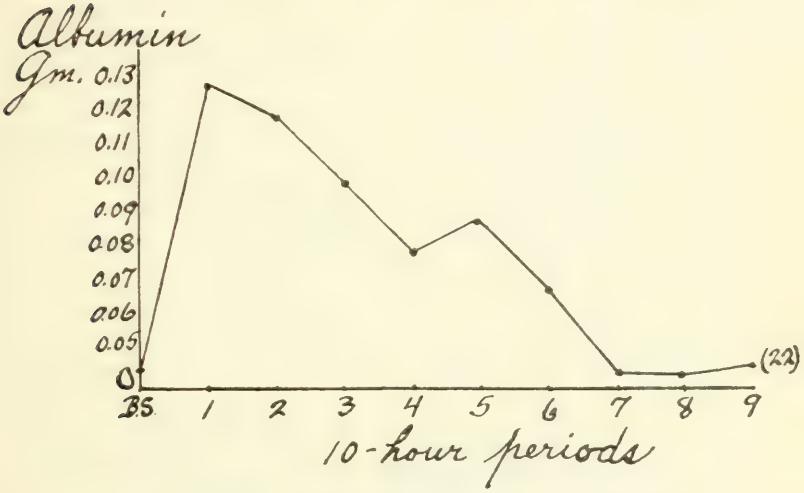

Fig. 1.—Excretion of albumin in urine in each ten-hour period following the administration of full therapeutic doses of salicylate. The curve represents median values; the number in parenthesis refers to number of different individuals; B. S. means "before salicyl."

from all the individuals studied (twenty-two) are presented in Table 2, and in Figure 1, which presents the course of the excretion by a curve of median values. This shows conclusively that a preexisting albuminuria is aggravated by the administration of salicyl. It will be seen, also, that the albuminuria reaches its maximum at, or shortly after, "toxicity," and gradually tends to reach its previous level. As judged from the

5. Folin and Denis: Jour. Biol. Chem., 1914, **18**, 273.

estimations of the salicyl content of these urines, the cessation of albumin excretion roughly coincides with the cessation of salicyl excretion. In a number of the individuals, however, the albuminuria tended to persist.

2. Diuresis Is Markedly Lessened.—As indicated by the curve in Figure 2 and the data in Table 3 (from twenty-five different individuals), the excretion of

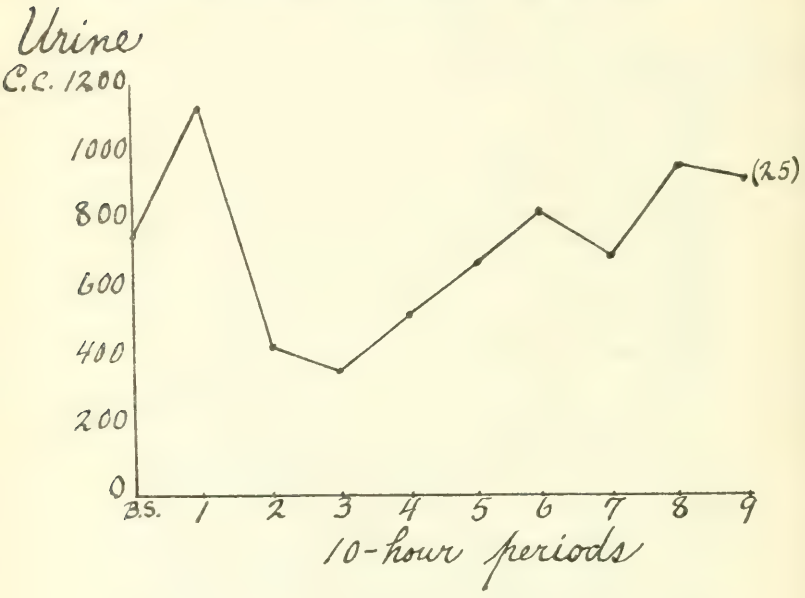

Fig. 2.—Excretion of urine in each ten-hour period following the administration of full therapeutic doses of salicylate. The curve represents median values; the number in parenthesis refers to number of different individuals; B. S. means "before salicyl."

urine is markedly diminished as a result of the administration of salicyl. The greatest depression is reached at, or shortly after, "toxicity," then the diuresis gradually increases and reaches practically its previous level about ninety hours after the administration of the drug. This diminution in water excretion is due, in part, to diaphoresis, which was usually marked, though

variable, in all the subjects; and, in part, to retention; that is, edema, which we shall describe in a forthcoming contribution.

3. Excretion of Phenolsulphonephthain Is Lessened.—The curves in Figure 3 and the data (from nineteen different individuals and twenty-two observations) in Table 4 show that there is a diminution in

TABLE 3. EXCRETION OF URINE BY INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE

No.	C.c. of Urine Excreted in Each Ten-Hour Period										
	B. S.*	1	2	3	4	5	6	7	8	9	10
1	....	1,720	1,123	703	428	404	1,000	1,858			
2	....	1,763	2,322	734	814	1,504	1,606	1,840	1,433		
3	....	2,001	446	330	274	343	325	638	943	864	791
4	....	2,153	1,094	1,307	1,884	1,586	1,586	2,080	1,642	2,144	2,073
5	....	1,074	714	579	634	400	662	464	1,491	1,191	928
6	....	3,239	469	1,315	899	1,071	978	708	670		
7	....	1,405	1,399	925	1,692	1,333	1,088	875	1,206	702	
8	....	1,970	786	1,359	1,549	1,695	1,478	1,562	2,264		
9	....	810	550	625	230	360	185	630	755	1,027	
10	....	855	210	425	530	840	810	460	550	350	1,275
11	....	595	420	175	495	290	263	455	375	387	392
12	....	640	340	660	680	690	1,130	776	1,420	960	970
16b†	295	1,510	375	122	360	655	575	440	962	338	558
16c	670	1,230	290	127	115	290	119	275	760	110	105
16d	760	760	205	350	.....	1,315	505	.....	2,305	1,510	
17†	410	1,015	455	270	380	750	460	310	400	430	430
18	760	1,150	225	170	170	250	540	339	365	410	540
19	695	1,105	352	320	265	220	305	505	860	1,030	510
20	180	1,140	250	270	310	230	1,165	154	740	410	475
21a	370	950	340	320	340	235	.....	525	.....	315	250
21b	950	1,380	425	250	980	790	950	1,450	700	1,700	730
22	1,425	1,070	450	640	910	920	930	1,140	1,560	1,100	930
23	900	630	495	220	680	620	750	.....	580	980	
24	580	695	325	335	250	310	440	270	660	300	250
25	700	1,605	250	380	870	1,275	620	1,250	985	950	
26	1,160	1,995	605	395	240	1,075	1,035	1,340	1,125	1,100	
27†	965	1,360	705	330	1,085	510	1,450	1,030	1,160		
28†	1,250	905	850	400	875	1,170	1,260	620	1,280		
Median	750	1,145	448	365	530	673	810	630	953	907	

* B. S. means "before salicyl."
† Received bicarbonate together with salicylate.

the two-hour excretion of phenolsulphonephthalein in the majority of persons after the administration of salicyl. This lasts for about twenty hours after administration is begun, and then gradually returns practically to its previous level. This diminution while relatively small, that is, about 10 per cent., is nevertheless definite, and quite constant in the majority of individuals.

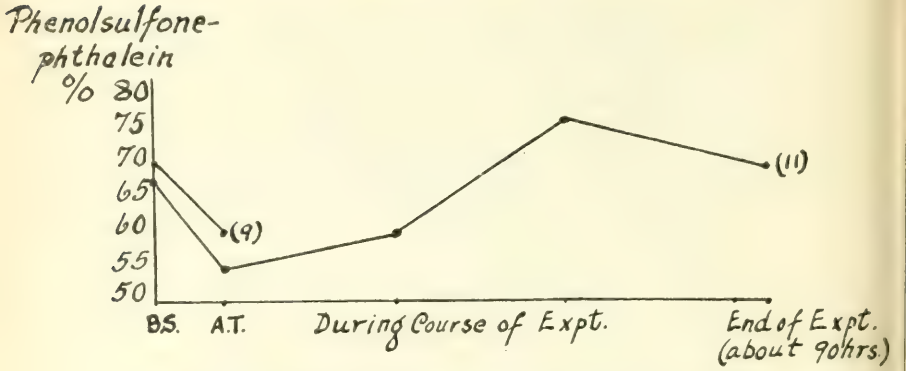

Fig. 3.—Excretion of phenolsulphonephthalein (two hours) in urine following the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity," that is, about eight to ten hours after administration; daily observations were made "during the course of the experiment"; the numbers in parentheses refer to number of different individuals.

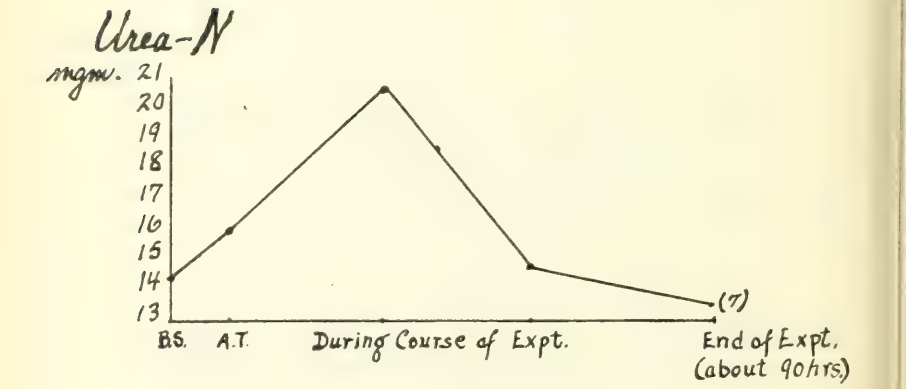

Fig. 4.—Accumulation of urea nitrogen of the blood (mg. in 100 c.c.) following the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity," that is, at the end of eight to ten hours after administration; daily observations were made "during the course of the experiment"; the number in parenthesis refers to number of different individuals.

TABLE 4.—EXCRETION OF PHENOLSULPHONEPHTHALEIN IN THE URINES OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE *

Number	Per Cent. of Phenolsulphonephthalein Excreted in Two Hours					
	B. S.	A. T.	Daily Observations After "Toxicity"			
			1	2	3	End of Experiment
6	60.0	55.0	60	..	..	60.0
7	55.0	60.0	55	..	..	60.0
8	70.0	60.0	65	..	..	70.0
16b†	30.0	35.0	..	..	..	35.0
17†	50.0	50.0	..	..	..	50.0
23	50.8	26.6	53	66	75	72.0
24	68.0	74.0	60	79	98‡	88.0
25	100.0	50.0	90	100	..	100.0
26	66.0	60.0	50	62	..	68.0
27†	89.0	75.0	76	94	..	90.0
28†	70.0	48.0	62	75	..	75.2
Median§	66.0	55.0	60	77	..	70.0
13	62.0	75.0				
14	70.0	50.0				
15	74.0	25.0				
16a	25.0	35.0				
16c	35.0	45.0				
16d	35.0	45.0				
18	60.0	60.0				
19	75.0	85.0				
20	75.0	75.0				
21	75.0	70.0				
22	75.0	80.0				
Median¶	70.0	60.0				

* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; the end of the experiment was about eighty to ninety hours after administration.

† Received sodium bicarbonate together with the salicylate.

‡ Mean of 98, 88, 95, 100, 97, 88, 100, 100 and 92.5 per cent. of nine following days, respectively.

§ Used in the construction of the long curve in Figure 3.

¶ Used in the construction of the short curve in Figure 3.

4. *Effect on the Nitrogenous Constituents of the Blood.*—Urea nitrogen: In the majority (seven) of persons in whom this was estimated, there was demonstrable a definite accumulation of urea nitrogen in the blood. This begins to be evident about the time of "toxicity," reaches its maximum about twenty-four to thirty hours after administration, and returns to its previous level at the end of about eighty to ninety hours (three and one-third to three and three-quarter days). This is shown by the curve in Figure 4 and the data in Table 5. These seven persons represent the most complete study of the nitrogenous constituents of the blood of our series.

TABLE 5. UREA NITROGEN CONTENT OF THE BLOOD OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE*

Number	Mg. Urea Nitrogen in 100 C.c. of Blood					
			Daily Observations After "Toxicity"			
	B. S.	A. T.	1	2	3	End of Experiment
22	6.4	4.3	....	....	....	6.0
23	11.5	14.3	15.6	14.0	16.0	10.1
24	9.8	12.5	20.5	14.7	13.5†	13.5
25	16.0	17.0	14.5	16.5	....	14.9
26	17.2	16.0	20.6	19.0	....	16.0
27‡	14.3	17.6	21.4	13.4	....	13.4
28‡	19.5	21.6	20.6	....	....	14.0
Median§	14.3	16.0	20.6	14.7	....	13.5
17‡	28.0	22.0				
16d	9.1	8.3				
18	9.1	6.0				
19	16.3	3.0				
20	7.4	7.6				
21	15.4	16.2				

* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; the end of the experiment was about eighty to ninety hours after administration.

† Mean of 12.3, 14.2, 12.1, 15.6, 13.5, 15.5, 12.5, and 13.5 for following eight days, respectively.

‡ Received bicarbonate together with salicylate.

§ Used for the construction of the curve in Figure 4.

In a number (six) of persons from whom it was impossible to obtain blood as frequently as was desirable, the results are not so conclusive, for the greatest accumulation of urea nitrogen would occur some time after the last specimen of blood was obtained. The blood was obtained only before the administration of the drug and at "toxicity." The results are presented in Table 5. It is seen there is a definite diminution of the urea nitrogen of the blood at "toxicity" in three (Patients 17, 18 and 19), and the content in the other patients was practically unchanged. It is quite likely that the important phase in the curve of accumulation of the urea nitrogen was missed in these experiments.

Nonprotein nitrogen: The results from nine different individuals are presented in Figure 5 and Table 6. These show a diminution in the nonprotein nitrogen content of the blood at "toxicity." This persists for a day or so, then gradually tends to return to its previous level at the end of the experiment, that is, about

eighty to ninety hours after administration. The different individuals showed some variations in these changes.

As to the possible explanation of this diminution in the total nonprotein nitrogen content of the blood, it is suggested that the uric acid fraction, being affected by the salicyl, is perhaps chiefly responsible. In our series no attempt was made to estimate the uric acid content of the blood, but this has been conclusively shown by Denis[6] to be diminished in the blood after the administration of salicyl in large doses, and at the same time the excretion in the urine is increased. In addition,

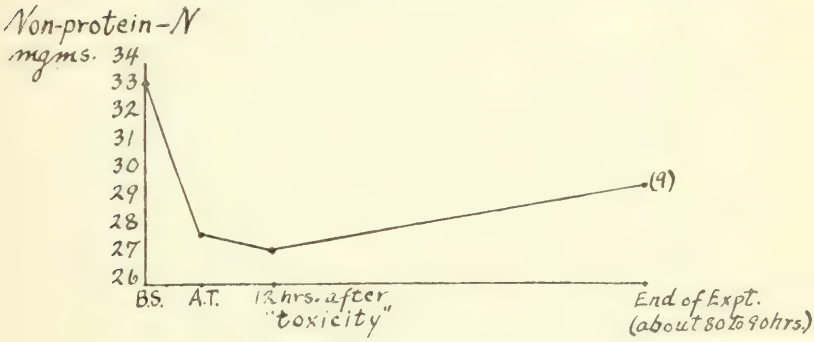

Fig. 5.—Total nonprotein nitrogen of the blood (mg. in 100 c.c.) after the administration of full therapeutic doses of salicylate. B. S. means "before salicyl"; A. T. means "at toxicity"; observations were also made twelve hours after "toxicity" and at the end of the experiment, that is, about eighty to ninety hours after administration; the number in parentheses refers to number of different individuals.

there are many observations by others showing definitely that salicyl increases the excretion of uric acid in the urine. The extent or degree of diminution in the total nonprotein nitrogen of the blood would to a certain extent depend on the content of the uric acid fraction before the administration of the drug, and in part on the functional state of the kidney. Because of this peculiar effect of the salicyl on the uric acid fraction, it appears that a study of the total nonprotein

6. Denis: Jour. Pharmacol. and Exper. Therap., 1915, 7, 255.

nitrogen content of the blood cannot serve as a reliable index of renal functional efficiency in individuals receiving large doses of the drug.

5. *Effect of Bicarbonate on the Albuminuria and Renal Functional Changes.*—It has been claimed (notably by Glaesgen[7] for human beings, and Frey[8] for rabbits) that the administration of bicarbonate together with salicylate, or rendering the urine alka-

TABLE 6. TOTAL NONPROTEIN NITROGEN OF THE BLOOD OF INDIVIDUALS RECEIVING FULL THERAPEUTIC DOSES OF SALICYLATE *

Number	Mg. of Nonprotein Nitrogen in 100 C.c. of Blood			
	B. S.	A. T.	12 Hours after "Toxicity"	End of Experiment
5	33.1	24.0	26.9	29.4
6	42.5	49.6	54.6	76.6
7	30.6	44.6	41.6	16.6
8	27.5	35.5	27.5	10.2
9	33.3	48.2	49.2	39.3
10	24.0	14.2	16.5	19.5
11	31.1	24.1	21.3	24.3
12	40.6	29.4	14.1	
16b†	35.1	20.9	....	43.3
16c	35.1	19.7	....	30.0
Median‡	33.2	27.8	27.2	29.4
12	66.3	63.1		
13	52.2	42.3		
14	52.2	52.5		
15	53.1	63.2		

* In this table, B. S. means "before salicyl"; A. T. means "at toxicity"; end of experiment was about eighty to ninety hours after administration

† Received bicarbonate together with salicylate.

‡ Used for the construction of the curve in Figure 5.

line during salicyl medication, tends to lessen or inhibit the albuminuria. Ehrmann,[9] who was not convinced that salicyl always caused albuminuria, claimed that alkalinity had no influence on the excretion of albumin in individuals in whom albuminuria occurred.

The results obtained by us do not support the claims that have been made for the beneficial effects of bicarbonate. Patients 13, 16b, 17, 27 and 28 received

7. Glaesgen: München. med. Wchnschr., 1911, **58**, 1125.
8. Frey: München. med. Wchnschr., 1905, **52**, 1326.
9. Ehrmann: München. med. Wchnschr., 1907, **54**, 2595.

bicarbonate, together with salicylate, and the majority showed albuminuria, and of about the same degree and duration, diminution in water and phenolsulphonephthalein excretion, and accumulation of urea nitrogen in the blood. The results for these may be seen in Tables 1, 2, 3, 4 and 5. The urines of Patients 13, 16b, 17 and 27 remained alkaline throughout the experiment, and of Patient 28 only about forty hours after the administration was begun.

Accordingly, bicarbonate has practically no demonstrable influence on the albuminuria and the changes in renal functional efficiency produced by the administration of salicyl in full therapeutic doses.

CONCLUSIONS

1. The administration of salicylate in full therapeutic doses invariably causes the appearance of albumin, white blood corpuscles and granular casts or cast-like bodies in the urines of normal, rheumatic, non-rheumatic, febrile and afebrile persons.

2. The albuminuria is not of febrile origin, but due directly to the drug.

3. A preexisting albuminuria is aggravated by the administration of salicylate.

4. So far as renal functional efficiency is concerned, there is a diminution. This is indicated by, (1) lessened water excretion [taken in connection with (2) and (3)]; (2) diminished phenolsulphonephthalein excretion, and (3) accumulation of urea nitrogen of the blood.

5. The administration of bicarbonate together with salicylate has practically no demonstrable influence on the albuminuria and renal functional changes produced by the salicyl.

THE SALICYLATES

VIII. SALICYL EDEMA *

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AND
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(*From Archives of Internal Medicine, September, 1917*)

Following the administration of full therapeutic doses of salicylate there is a rather marked diminution in urine output, reaching its greatest depression about ten to twenty hours after the symptoms of toxicity appear and persisting for about forty to seventy hours after the administration of the drug. The output of urine reaches its previous level roughly about the time excretion of salicyl is completed.

Two possible explanations are suggested for this: (1) sweating; (2) retention of water, that is, edema; the important factors to be considered in retention are (*a*) tissues, and (*b*) kidney.

It is the object of this communication to report certain facts bearing on these causes. The work was conducted in a quantitative way and on persons some of whom were practically normal, others convalescent from various disorders. The following procedures were carried out before and after the administration of the salicylate, and throughout the experiment, which usually lasted about a week. The persons were weighed regularly, about three times in ten hours, and the drug was not administered until the weight curve became practically constant. This was reached usu-

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* This investigation was supported, in part, by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

ally within two to three days after the person was put to bed and placed on the regular routine. The water and dietary intakes were maintained as constant as possible throughout the procedure. Two hundred c.c. of water were administered every two hours. Coffee or milk was substituted for this at meal times. The meals were taken at the same hours, and the quantity was constant throughout. The administration of salicyl was so timed that the symptoms of toxicity would

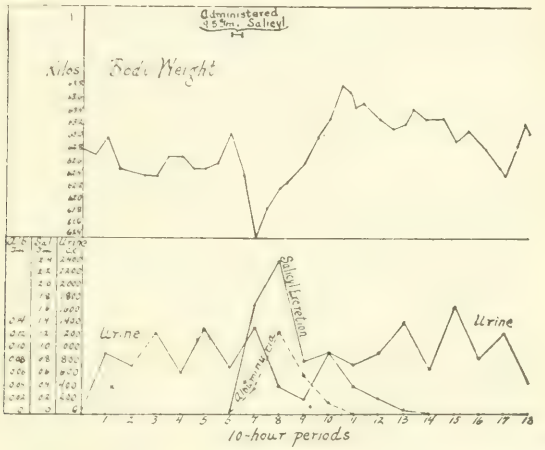

Chart 1.—Patient 21b (J. V.). In this and all the other charts the brace at the top of the chart indicates the period during which salicyl was administered; salicyl refers to salicylic acid; Alb, to albumin; sal, to salicyl; the cross ×, to the quantity (grams) of feces voided; 'phthalein refers to phenolsulphonephthalein.

come when the stomach would be empty so as to avoid loss of contents in case emesis should occur. No attention was paid to the qualitative nature of the dietary, but even in this there were no radical changes, since the food in this hospital is remarkably constant in quality at all times.

Renal function was studied by the output of urine, which was collected in ten-hour periods; the quantitative excretion of albumin (method of Folin and

Denis[1]); and daily observations on the phenolsulphonephthalein excretion (two-hour, and carried out in the usual way) and urea nitrogen of the blood (by the urease, aeration and colorimetric procedure). Hemoglobin estimations were made with the idea of ascertaining the chief depot of the water retention, that is, whether the blood or tissues. For this, the

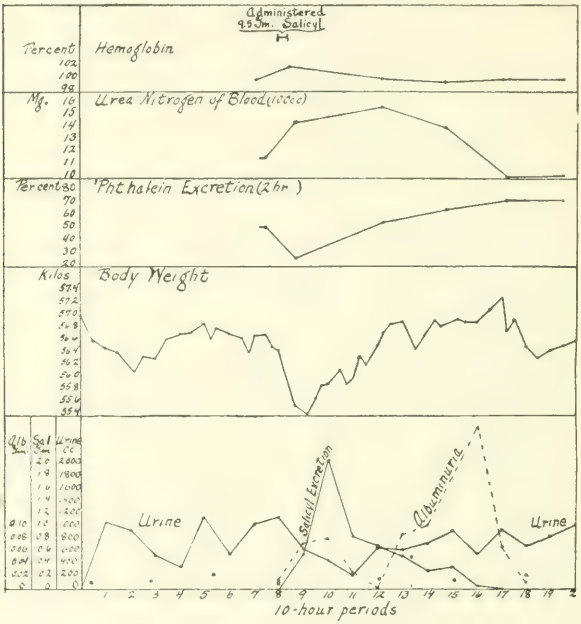

Chart 2.—Patient 23 (J. E.). Marked diaphoresis occurred from end of ninth to twelfth periods, inclusive.

carbon monoxid colorimetric method of Haldane[2] was chosen, using the Duboscq colorimeter for making the readings instead of test tubes. The results are expressed as relative percentages of hemoglobin. The effect on and the rôle of the tissues were also judged, in part by the course of salicyl excretion in the urine.

1. Folin and Denis: Jour. Biol. Chem., 1914, **18**, 273.
2. Haldane: Jour. Physiol., 1901, **26**, 497.

The salicyl was estimated according to a method previously described.[3] Sweating was judged, in part, from the weight curve and, in part, by direct inspection and the subjective symptoms reported by the patient himself.

When the weight and urine curves became practically constant, the salicylate was administered. A

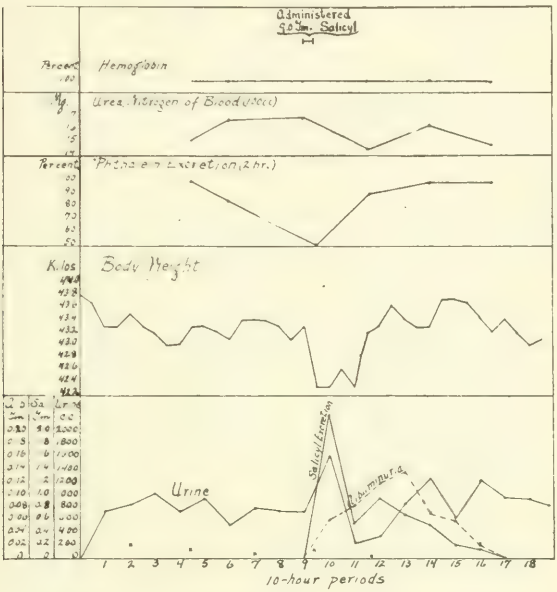

Chart 4.—Patient 25 (H. V.). Mild diaphoresis occurred from end of eleventh to thirteenth periods, inclusive.

definite quantity (20 c.c.) of sodium salicylate (usually about 10 per cent.) was administered together with 80 c.c. of water every hour until toxicity, when the administration was stopped. The water intake was then maintained at the rate of 200 c.c. every two hours, and the various observations according to the procedures detailed above were carried out until the

3. Thoburn and Hanzlik: Jour. Biol. Chem., 1915, **23**, 163.

TABLE OF CLINICAL DATA

Number and Patient	Diagnosis	Fluid and Dietary Intake	Total Quantity of Salicyl* Administered (gm.)	Diaphoresis	Remarks
21b (J. V.)	Normal; recovered from rheumatic fever	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	9.5	Imperceptible	
23 (J. E.)	Synechia cordis; chronic myocarditis	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	9.5	Marked	
24 (J. H.)	Chronic endocarditis and myocarditis	100 c.c. every hour for 5 hours 200 c.c every 2 hours thereafter 320 gm. food 3 times daily	8.1	Mild	
25 (H. V.)	Catarrhal conjunctivitis	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	9.0	Mild	
26 (R. H.)	Corneal ulcer (left eye)	100 c.c. every hour for 7 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	12.6	Marked	
27 (J. M.)	Varicose ulcer of leg.....	100 c.c. every hour for 7 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	12.6	Mild	14 gm. $NaHCO_3$ administered together with salicyl
28 (T. S.)	Alcoholic gastritis.....	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	8.01	Moderate	9 gm. $NaHCO_3$ administered together with salicyl
29 (L. K.)	Rheumatic fever (mild attack)	100 c.c. every hour for 5 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	8.9	Marked	8 gm. $NaHCO_3$ administered together with salicyl
30 (H. M.)	Chronic arthritis....	100 c.c. every hour for 8 hours 200 c.c. every 2 hours thereafter 384 gm. food 3 times daily	14.24	Just perceptible	16 gm. $NaHCO_3$ administered together with salicyl

* Expressed as salicylic acid.

end of the experiment. In all, nine persons have been studied. Four of these (Patients 27, 28, 29 and 30) received sodium bicarbonate together with the salicylate. The detailed protocols for each individual are so long that they have been omitted entirely, and instead the data are presented in the form of curves for each one. Various clinical data pertaining to all the individuals are presented in the accompanying table.

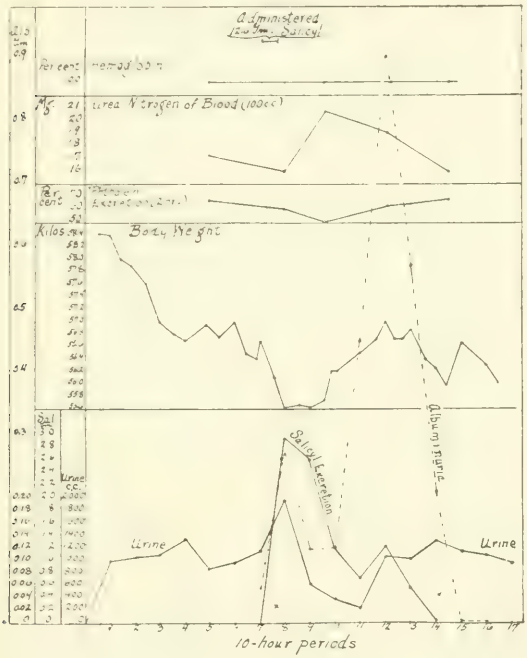

Chart 5.—Patient 26 (R. H.). Marked diaphoresis occurred from the ninth to end of eleventh periods, inclusive.

1. *Salicylate Causes Edema.*—This is indicated by the fact that there is an increase in body weight demonstrable after the administration of the drug, unless this is prevented by sweating. Charts 1, 2, 3, 4, 6, 7 and 9 indicate definite increases in body weight and above the level before the drug was administered.

This increase ranges from moderate to considerable. The diaphoresis ranged from imperceptible to just perceptible, and roughly, inversely proportional to changes in the body weight. That is, a relatively small diaphoresis is accompanied by a more marked increase in body weight.

Charts 5 and 8 show practically no changes in body weight. These persons gave evidences of marked diaphoresis. So far as other phenomena are concerned,

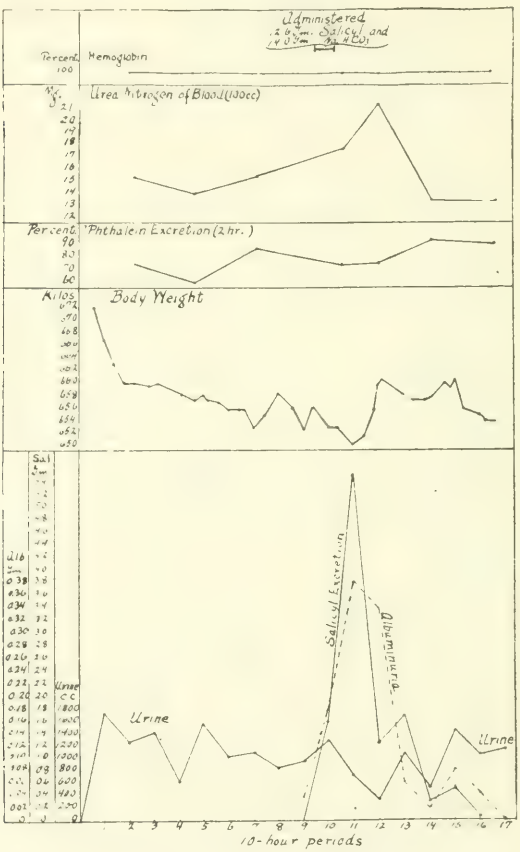

Chart 6.—Patient 27 (J. M.).

these remained unchanged. In nearly every case there is evidence of a sharp decline in body weight about the time of toxicity. This is due, no doubt, to diaphoresis which occurs at this time and when the concentration of the drug in the tissues is at its maximum. This, of course, is a well known action of salicylate. Persistent sweating in those persons who habitually sweat considerably resulted in no modifica-

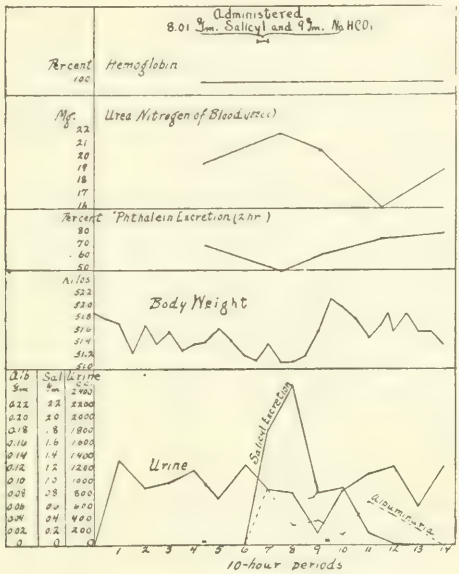

Chart 7.—Patient 28 (T. S.).

tion of the curves of body weight. The loss of body weight cannot be attributed to increased urine or fecal output, for these do not adequately compensate for the losses. The respiratory rate remains unchanged. The loss of water, therefore, could only have taken place by evaporation, that is, by diaphoresis.

It may be concluded that the administration of salicylate in full therapeutic doses causes a relative anuria, and this is due to retention of the water, that is, edema,

as indicated by an increase in body weight unless modified by sweating.

2. *The Water Retention Is Not Due to Retention of Salicyl in the Tissues.* This is indicated by the curves of salicyl excretion[4] in all the figures, which represent the data obtained for different individuals. It is seen that the maximum excretion of salicyl occurs at a time when the body weight begins to increase and when the water excretion is lowest. The excretion is at its minimum when the body weight is at its maximum; that is, when the water retention or edema is most marked, and in most individuals this was completed before the body weight reached its previous level. The excretion of salicyl does not go parallel with the curve of body weight. There is no retention of salicyl and the retention of water, therefore, is not associated with or due to retention of salicyl in the tissues.

3. *The Water Is Not Retained by the Blood.*—This is indicated by the curves of hemoglobin percentage of the blood. Dilution of the blood was demonstrable in only one patient (24, Chart 3), in whom for a time there was some cardiac decompensation. On the other hand, an increase in the percentage of hemoglobin at toxicity was demonstrable in two patients. These persons sweated considerably from which it is inferred that there was some concentration of the blood at this time. In all other patients, whose blood was studied, no changes in blood volume were demonstrable.

From these evidences it appears logical to conclude that the water is retained in the tissues.

4. *The Retention Is Chiefly of Renal Origin.*—Previous studies[5] on renal function showed that the administration of salicylate impairs the functional efficiency of the kidney. This also is the case in the persons studied in connection with edema. Indeed it is one of the most constant features of the experi-

4. For other studies on the quantitative excretion of salicyl consult an earlier publication by Hanzlik, Scott and Thoburn: Jour. Pharmacol. and Exper. Therap., 1917, **9**, 247, and in connection with retention, Jour. Pharmacol and Exper. Therap., 1917, **9**, 217.

5. Hanzlik and Karsner: Arch. Int. Med., 1917, **19**, 1016. Hanzlik, Scott and Thoburn: Ibid., p. 1030.

ments, and is indicated in the various curves by a diminution in phenolsulphonephthalein excretion and at the same time an accumulation of urea nitrogen of the blood. Albumin, leukocytes and casts also

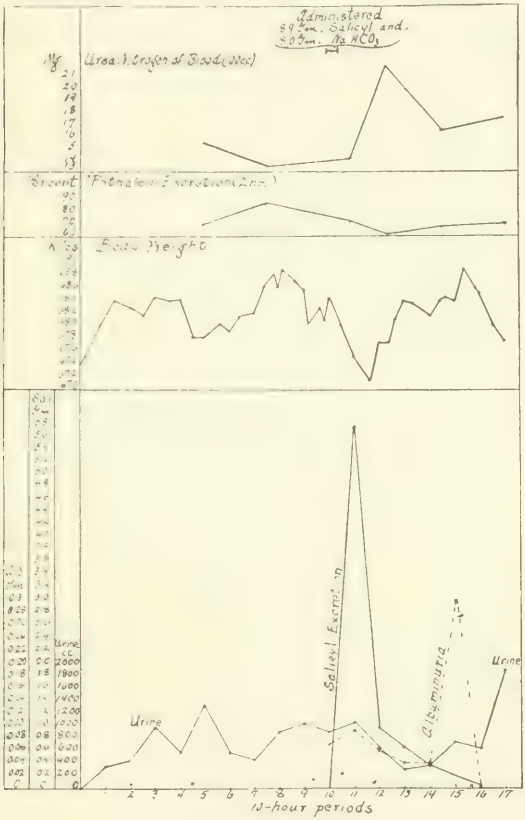

Chart 8.—Patient 29 (L. K.).

appeared in the urines. This diminution in renal functional efficiency is demonstrable about the time of toxicity, gradually becomes more severe, and finally (end of eighty to ninety hours) returns to its previous state. In almost every instance the impairment in

renal function appeared before an increase in body weight was demonstrable. From this it seems that the renal factor plays an important rôle in the production of edema. It must be admitted that the part which tissues in general might play have not been abso-

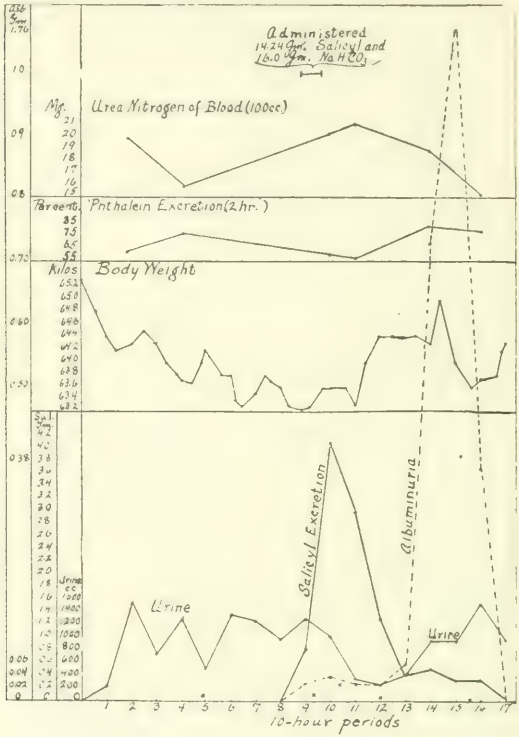

Chart 9.—Patient 30 (H. M.).

lutely excluded, for it is conceivable that in the beginning a marked diaphoresis could compensate for any accumulation of water.

However, the following observation made by Sollmann and Pilcher[6] tends to exclude direct action on

6. Sollmann and Pilcher: Jour. Pharmacol. and Exper. Therap., 1917, **9**, 309.

the tissues, or at least so far as local edema is concerned, and therefore lends support to the renal factor. In connection with the study of a series of agents which give rise to endermic reactions characterized by hyperemia, swelling (wheal formation), etc., the application of sodium salicylate to the abrazed skin does not give rise to local edema or urticaria such as is unmistakably produced by a large number of other drugs. The concentration used by Sollmann and Pilcher is much higher than the concentration of salicyl we have found in the blood even after the largest doses used by us. There is no reason to believe that salicyl would be more effective in smaller than in larger concentrations.

5. *Effect of the Administration of Sodium Bicarbonate.*—If the edema produced by the administration of salicylate is of the same nature as the salt edema of Widal, it could conceivably be accentuated by the administration of such a salt as sodium bicarbonate. It has also been observed that the administration of large doses of bicarbonate can cause edema. This also gave us the opportunity to test the claim that is made by some, namely, that edema is due to the production of acid, and the rational therapy indicated consists of the administration of alkali.

Sodium bicarbonate together with salicylate was administered to Patients 27, 28, 29 and 30. The urines remained alkaline throughout the experiment, or at least, during the increase in body weight. The phenomena observed under the bicarbonate-salicylate medication as compared with salicylate alone, remained practically unaltered. Of four patients studied, only one (29, Chart 8) failed to show an increase in body weight, owing to marked diaphoresis.

Sodium bicarbonate, therefore, has no demonstrable influence on the edema and diminution in renal functional efficiency, albuminuria, anuria, etc., produced by the administration of full therapeutic doses of salicylate.

CONCLUSIONS

1. The anuria produced by the administration of full therapeutic doses of salicylate is due to retention of water as indicated by an increase in body weight unless modified by diaphoresis.

2. This retention is demonstrable about twenty hours after the start of administration of the salicylate and persists until the salicyl excretion is completed, that is, about eighty hours (three and one-third days).

3. The retention occurs chiefly in the tissues, for no dilution of the blood is demonstrable by estimations of hemoglobin.

4. The edema is accompanied by a diminution in phenolsulphonephthalein excretion together with an accumulation of urea nitrogen of the blood, and increased excretion of albumin, all of these elements reaching their previous levels with the disappearance of the edema.

5. There is, therefore, a diminution in renal functional efficiency, and since this generally makes its appearance before an actual increase in body weight is demonstrable (edema) and later coincides with it, it seems that the renal factor plays an important rôle in the production of the edema.

6. These phenomena are not modified by the administration of sodium bicarbonate together with the salicylate, and in doses sufficient to maintain the urine alkaline.

We wish to express our thanks to Supt. C. H. McFarland and Dr. E. P. Carter, Chief of Medical Service, for extending the use of a special ward without which this and other investigations on salicylate would have been impossible; to Drs. Sheets and Kennedy, interns, and Messrs. T. W. Thoburn, M. E. Fulk and J. A. West of the third year class for various services rendered in connection with the work.

PIPERAZIN AND OTHER ORGANIC URATE SOLVENTS*

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CLEVELAND

(*From the Journal of Laboratory and Clinical Medicine, February, 1917*)

In a previous paper on hexamethylenamine (Hanzlik, 1916) a review of the literature revealed the practically negligible position of this drug among the so-called urate solvents, particularly when contrasted with such well known and tried alkalies as the bicarbonate and citrate of sodium. Special urate solvent properties have been ascribed to other agents. Among the best known and widely exploited, of these is piperazin. However, our knowledge of the behavior of uric acid and urates in the body and its fluids makes it difficult to understand why any special virtues should be ascribed to this product.

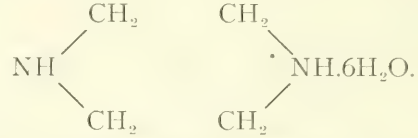
It is the object of this paper to examine the literature on piperazin and other urate solvents and to ascertain what, if any, scientific data exist to support the claims that are made. A proper appreciation of the chemical properties and the behavior of uric acid and the urates in the body is necessary in order to judge the data. For this the previous paper may be consulted. The complexity of the chemical behavior of the urates is further illustrated in recent contributions by Lambling and Debaussy (1914) and Haskins (1916). These emphasize the paradoxical behavior of the solvent power of urine itself for uric acid, and other properties, rendering positive deductions extremely difficult. It may be further stated that positive and optimistic statements regarding the therapeutic efficiency of the class of substances called urate

* From the Pharmacological Laboratory, Western Reserve University, Cleveland. This review was prepared at the request of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

solvents should be generally regarded as premature and unfounded, particularly if it is remembered that the clinical conditions for which these are frequently recommended are still more obscure. Bearing these uncertainties in mind, we may now proceed to examine the possibilities offered by piperazin as a urate solvent.

1. ADDITION OF PIPERAZIN TO PURE URATE

Piperazin is a synthetic organic base obtained by the condensation of two ethylene with two amino groups. It forms soluble salts with uric and other acids. Its structural formula is



Solubility of Piperazin Urate.—Piperazin dissolves uric acid even in the cold, 1 part of the salt, piperazin urate, being soluble in 50 parts of water at 17° (Mayer and Schmidt, 1890). According to Mathews (1915) the piperazin salt of uric acid is much more soluble than the alkaline salts, the solubility being about 1 in 50 parts of water at 17°. Biesenthal and Schmidt (1891) give the solubility of various urates in water as follows: Piperazin urate 1 : 50; acid sodium urate 1 : 1,200 at 15°; lithium urate 1 : 367 at 20°. Vicario (1902) reports that 1 part of piperazin urate is soluble in 45 parts of water at 18°, in 44 parts at 37°; 1 part of acid sodium urate in 1,136 at 18°, and in 581 parts at 37°. This is less than reported by Vicario for sodium urate, which was found to be 35 parts at 37°; somewhat higher, 59 parts, at 18°. One part of potassium acid urate was soluble in 666 at 18°, and 345 at 37°. Helbing and Passmore (1894) claim that piperazin can form an acid salt with uric acid, but that this never occurs in therapeutic practice. They claim further that neutral piperazin urate is the normal urate produced by the solvent action of piperazin on uric acid. It is said to be extremely soluble, 1 part piperazin urate dissolving

in 50 parts water at 17°, in 40 parts at 38°. These data agree favorably with those reported by others as pointed out above. In attempting to compare the solubility of uric acid in piperazin in low or therapeutic concentrations, Helbing and Passmore worked with percentages that are altogether too high. They state that the concentration of 0.025 per cent. would approximately represent the percentage of piperazin in the blood after a dose of 15 grains (about 1 gm.). However, it is not correct to assume the distribution of the drug to be limited to the blood only. The tissues as a whole should be considered. The weight of the individual is not stated, but assuming it to be an adult of about 70 kgs., the concentration in the tissues, as a matter of fact, would be about 0.001 per cent., and 0.002 per cent. for a 50 kg. individual.

Comparison With Lithium and Effect of Neutral Salts.—In test-tube experiments, according to Cushny (1910) and Schmiedeberg (1909), the solubility of uric acid in piperazin is greater than that of lithium and borax. Heinz (1907) states this to be about twelve times that of lithium carbonate; Helbing and Passmore (1894) about seven times. This is denied by Van der Klip (1892), who found the solvent power of piperazin (concentration unknown) to be less than that of lithium carbonate between 16° and 36°. Plugge (1895) states that it is only in high concentrations that piperazin is a better uric acid solvent than lithium. In dilute solutions it is less so. Ortowski (1900) found that piperazin in aqueous solution at 37.5° possessed less solvent power for uric acid than lysidin, but acted somewhat more favorably than sodium bicarbonate.

In the presence of even small quantities of sodium chlorid (less than 1 per cent.) this solubility is lessened, hence in urine the solvent power of piperazin is almost completely lost. Morhorst (1896) states that the addition of sodium chlorid, sodium sulphate and other neutral salts to a piperazin solution of uric acid causes precipitation of urate from the piperazin urate. According to Penzoldt (1900) the solvent power of aqueous solutions of piperazin is lessened by

the addition of urine. He found that a uric acid stone placed in a 1 per cent. solution of piperazin and incubated for twenty-four hours was dissolved along the edges only. Nicolaier (1899) thought that piperazin dissolved uric acid and urates less readily than hexamethylenamine. On the other hand, Stevens and May (1911) found that piperazin was a better solvent than hexamethylenamine.

It may be concluded that piperazin forms a salt with uric acid called piperazin urate, whose solubility is about 1 in 50 parts of water, and that this is diminished by the addition of urine and weak concentrations of neutral salts. Lithium apparently is a better solvent for uric acid in vitro than piperazin in low concentrations.

2. ADDITION OF PIPERAZIN TO URATE CALCULI

Casper (1897) observed that as solvents for calculi piperazin, lysidine and hexamethylenamine were no more effective than urine or water alone, but glycerin was superior. Biesenthal and Schmidt (1891) observed that 1 per cent. piperazin dissolved a urate stone better than lithium carbonate, sodium carbonate, and borax of the same strength. Gordon (1894) treated natural vesical calculi with piperazin, borax, sodium bicarbonate, lithium citrate and potassium citrate. One per cent. piperazin was found to possess considerable solvent power if the contact was long enough. The calculi were softened, rendered mushy and it was conceived that in the body they would be somewhat more easily "absorbed." When 50 mg. of a powdered stone consisting of 59.19 per cent. uric acid was placed into 1 per cent. piperazin for twenty-four hours at 39°, 48 mg. were dissolved while the other solvents under the same conditions dissolved only 25 to 35 mg. This led to the conclusion that the solvent power of piperazin under like conditions is greater than that of borax, lithium citrate, sodium bicarbonate or potassium citrate. On the other hand,

Van der Klip (1892) found that uric acid calculi are not dissolved by piperazin as readily as by lithium carbonate.

Ebstein and Sprague (1891) used piperazin as a solvent in the course of analytic procedure and found that gouty tophi consisting of urate can be dissolved by 1:1,000 piperazin in vitro. Finzelberg (cit. Helbing and Passmore, 1894) is said to have made experiments demonstrating the solvent power of piperazin for different kinds of stone, and from this he suggested the use of the drug in the treatment of arteriosclerosis, "compound" sclerosis and atheromatous changes of the aorta.

It may be concluded that piperazin, in rather high concentration, possesses some power to dissolve calculi, but the extent of this may be regarded in some cases limited and doubtful, due in part, no doubt, to the uncertain and variable composition of calculi.

3. SOLUBILITY OF URIC ACID AND URATE IN SERUM CONTAINING PIPERAZIN

The existing scientific data, though limited, indicate this to be of no practical importance.

Sir W. Roberts (Croonian Lectures, 1892) stated that the addition of 0.1 to 0.2 per cent. piperazin to blood serum or synovial fluid had no effect in increasing the solvent power of these media on sodium biurate, nor did it retard the precipitation of uric acid from serum and synovial fluid.

Bohland (1894) found that 50 c.c. of serum (horse and calf) containing 1 per cent. piperazin dissolved about 0.1 gm. uric acid at ordinary room temperature, that is, about 0.2 per cent. From this solution (on standing), or when serum is added to an aqueous solution of piperazin, a flocculent precipitate is formed consisting chiefly of biurate. The solubility of potassium acid urate in 1 per cent. piperazin was found to be the same as for uric acid. Tunicliffe and Rosenheim (1898) found the solubility of sodium biurate in beef serum to be 1:31,000; other solvents in order of increasing efficiency being lysidine 1:25,000; hexa-

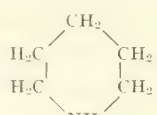
methylenamine 1 : 14,000; and piperidin[1] 1 : 12,000. The solubility in serum alone was found to be 1 : 60,000, which, if given the value of 1, would give piperazin a value of 1.9. The true reaction of the serum in all these experiments is not indicated, and as it might conceivably become strongly alkaline (by decomposition) the value of the results is considerably minimized.

4. SOLVENT ACTION OF PIPERAZIN URINE ON ADDED URIC ACID

The solubility of uric acid in piperazin urine is much less than in aqueous solutions and it is markedly influenced by dilution and the reaction. Unfortunately the effects of dilution and reaction have been overlooked by a majority of the earlier observers.

Helbing and Passmore (1894) found that urine interferes with the urate solvent action of piperazin. They observed that 100 c.c. of 1 per cent. piperazin urine dissolved only from 0.0065 to 0.03 gm. of uric acid in seventy-two hours; 100 c.c. of 0.1 per cent. piperazin urine dissolved from 0.0004 to 0.0017 gm. uric acid in seventy-two hours. Goodbody (1896) observed that the addition of 0.1 gm. piperazin to 220 c.c. urine, 0.2 gm. in another, which regularly deposited urate, dissolved the urate to the extent of 0.01 per cent. The average uric acid content of five urines before the addition of piperazin was 0.058 per cent., and this was increased to 0.067 per cent. by the addition of 0.2 gm. of the drug. The addition of piperazin (0.085 to 0.68 per cent.) to urine (100 c.c.) containing added uric acid caused variable quantities (28.12 to 233.32 mg., respectively) of the latter to go into solu-

1. Piperidin is a rather active substance pharmacologically, causing a rise of blood pressure in cold-blooded animals, cardiac inhibition with large doses, and a curare-like action on motor nerve endings. According to Oesterle (1909), the formula is



tion (Haskins, 1916). This was also true of dilute and less dilute urines after the administration of the drug and whether the reaction was truly acid or alkaline. Dilute urines without the drug but possessing the same reactions frequently dissolved more uric acid than when they contained piperazin. Generally, alkaline urines dissolved more uric acid, but it is also true that Haskins found considerable uric acid dissolved in a few acid urines. After the administration of 1 to 2.5 gm. daily to two individuals during the course of three days, Heubach (1891-92) observed that the piperazin urine would not dissolve any added uric acid even at higher temperatures and with prolonged action. Meisels claims that 70 per cent. of added uric acid (presumably to urine) was dissolved at room temperature. However, complete data are not cited in the report which was available. Mendelsohn (1892) could demonstrate no solvent action on calculi or uric acid in piperazin urines and concluded from this that no better results could be hoped for from injections into the bladder, because such solutions can be retained for two to three hours only, and the solvent effect would decrease with the advent of urine, rendering the solvent action of piperazin practically useless. Penzoldt (1900) states the uric acid solvent property of aqueous solutions of piperazin are lessened by the addition of urine. Tunicliffe and Rosenheim (1898) found that 100 c.c. of a 0.2 per cent. piperazin urine dissolved 0.058 gm. or 11.6 per cent. of 0.5 gm. of added uric acid; 100 c.c. of 0.1 per cent. piperazin urine dissolved 0.0415 gm. or about 8.3 per cent. Urine alone dissolved extra uric acid, and if the value of 1 is assigned to urine, the value for 0.2 per cent. piperazin would be about 13.5, for 0.1 per cent. it would be 5.7. Piperidin and lysidine were found to be more effective, hexamethylenamine less efficient. Weiss (1900) states that urates of piperazin and lysidine are soluble in water, but not in urine, an objection that cannot be held against urosin (to be considered later).

5. EFFECT ON URATE DEPOSITS

Experimental Deposits in Birds.—Deposits in the various organs of birds produced by foreign substances have been regarded by some investigators as the clinical equivalent of urate deposits in various disease conditions in human individuals, and gouty tophi. It is apparent that the conclusions drawn from such uncertain analogies furnish a very insecure basis for the therapeutic efficiency of the so-called urate solvents in gout and similar conditions. Nevertheless, we may briefly allude to the effects of piperazin in this interesting avian condition.

Fawcett (1894) observed that deposits produced in pigeons by gradual injections of chromate disappeared after the injection of 0.001 to 0.005 gm. piperazin. The kidneys were found to be swollen, pale or mottled, and Fawcett suggests that as a result of the piperazin injections the outlet for uric acid was blocked and the uric acid "deposited" in the tissues. Presumably retention of uric acid rather than actual deposits was intended. Practically the same was observed by Meisels (1893), who found that not only could piperazin prevent the formation of deposits but also remove those already formed. Whereas control animals showed a parenchymatous nephritis and uric acid concrements in the tubules, those treated with piperazin were free from these. Lithium carbonate and sodium bicarbonate were found to be without any influence on urate deposits in birds. Waucomont (1912) produced experimental gout in chickens by the feeding of horse flesh, and observed the effects of various urate solvents on this. It was found that piperazin, lycetol, sidonal and potassium iodide produced no influence on the excretion of uric acid, and most of these agents also did not influence the course of the clinical condition. The disappearance of joint swellings, deposits and symptoms of gout in an 8 year old parrot following the administration of piperazin (0.08 gm. daily) is reported by Zimmermann (1901). However, since the patient received bicarbonate internally, and salicylated collodion locally together with the piperazin treatment, it cannot be concluded which,

if any, of the beneficial effects were due to piperazin alone. Biesenthal (1893) claims to have confirmed the work of Meisels (1893), and thereby procured support for the therapeutic claims made for the drug in "uric acid diathesis." Both chickens and pigeons were used. About 7 per cent. of the animals injected with chromate failed to respond with demonstrable urate deposits. Eighty per cent. (sixteen) of the chickens and 82 per cent. (fourteen) of the pigeons treated with piperazin showed no deposits. However, since a considerable number of animals fail to give evidence of deposits with repeated injections of chromate, a large element of uncertainty must exist in this form of experimentation, likewise in the conclusions drawn therefrom. Proportionately for human individuals, the doses used by Biesenthal were very large, a total dosage of 27 gm. having been administered. Lithium carbonate, sodium phosphate and borax are reported by Biesenthal not to have influenced the deposits.

Deposits in Gout and Other Conditions.—According to Meyer and Gottlieb (1914) the view that piperazin and lysidine cause removal of retained uric acid in the tissues has been found to be erroneous. Mordhorst (1892) observed that piperazin would be a good solvent for urates, but a 1 per cent. solution, which is effective, is inconceivable in the body, the concentration in blood being about 0.0125 per cent. A disappearance of uric acid crystals in standing urine was noticeable, but the same followed more completely with the use of mineral waters. Mordhorst points out that the excretion of piperazin after internal administration, its destruction in part, and the doubtful affinity displayed by piperazin for uric acid being greater than that of the alkaline salts of the blood are important additional reasons why such agents as piperazin and lysidine cannot act favorably on urate deposits in the living organism. In a later communication, Mordhorst (1896) emphasizes the absurdity of expecting the alleged solvent effects of piperazin. He states that piperazin cannot act in the living organism because of the homeopathic concentration of the drug

in the body, about 1 : 54,000, and it must be remembered that a ½ per cent. solution dissolves uric acid very little, ¼ per cent. still less and ⅛ per cent. not at all. "It is therefore not understandable how anyone can earnestly believe that a solution of this salt, 1 : 54,000, can have even the slightest uric acid solvent action." Yeo (1909) quotes Sir Roberts to the effect that both piperazin and lithium cannot prevent the formation of uratic deposits.

6. EFFECT OF THE ADMINISTRATION OF PIPERAZIN ON URIC ACID AND URATES

According to Haskins (1916), who has furnished the best scientific evidence on this subject, piperazin in small doses does not affect the excretion of uric acid in urine. Using proper controls and carefully observing the reaction of the urine (hydrogen-ion concentration), Haskins found that when piperazin is administered in very large doses only (extratherapeutic) there is some increase in the excretion of uric acid. However, this was more effectively produced by the administration of ordinary alkalies such as citrate and bicarbonate of sodium. The use of large doses of piperazin according to some clinical reports (to be referred to later more fully) is perhaps harmful and, therefore, not justified, particularly in view of the fact that the same object may be attained by the use of well tried and relatively harmless alkalies. The work of Haskins is confirmatory of certain of the older observers, although it must be admitted that there is an element of uncertainty in their work owing to the lack of proper dietary and other controls, the failure to realize the importance of the reaction of urine, the influence of diuresis and the variable solvent power of urine itself. An exception of the older work is that of D. D. Stewart, and it agrees essentially with that of Haskins.

D. D. Stewart (1893) showed quite conclusively that medium-sized doses of piperazin used over long periods are without effect on uric acid excretion. This observer carefully studied the volume of urine, specific gravity, acidity, urea and uric acid excretion

daily before and after administration of the drug for several days in three cases of renal stone, using also proper fluid intake and, apparently, dietary controls. The dosage of piperazin was about 24 grains (1.5 gm.) daily for fourteen days in each case. Under these conditions there was no increase in uric acid elimination demonstrable and attributable to the drug, also no increase in the urea excretion. Later observations by Stewart (1894) with even larger doses of the drug, 70 grains (nearly 5 gm.), daily, confirmed his previous results. That is, the excretion of uric acid, urea and chlorid remained uninfluenced.

The following abstracts are cited against piperazin as a urate solvent: According to S. Fraenkel (1912) objective experiments indicate that the uric acid solvents, such as piperazin and others pertaining to its class, are worthless and when effects are seen, these are produced more by other conditions. Percy May (1911) is of a similar opinion, namely, that while some benefit may be derived from the administration of the piperazin class of urate remedies, this is not due to their solvent action on the uric acid. The employment of these bases as uric acid solvents is fallacious because there is always sufficient sodium present in the body to form the sparingly soluble sodium urate. Further, owing to the detrimental effects of small quantities of sodium chloride on the solvent power of piperazin, and the fact that it is to a large extent destroyed in the body, leaving only a small quantity unchanged in the urine, the uric acid solvent action of the drug on internal administration seems very problematic (Heinz, 1907). According to Cushny (1910), the urine of patients receiving piperazin has no more solvent action on uric acid than urine itself; and whatever piperazin escapes into the urine is in combination with the stronger acids and not uric acid. Hare (1905) states that repeated clinical observation has shown that the administration of the drug causes an increase in the amount of urea in the urine with decrease in uric acid indicating that under its influence oxidation is more complete. He gives no data. Various conditions in which piperazin is used and recom-

mended are, in Hare's experience, not benefited by the administration of the drug. Likewise Penzoldt (1900) claims he could recognize no influence by piperazin medication on the decrease of uric acid excretion. Poullsson (1912) doubts (for well known reasons, previously mentioned), whether piperazin has ability to dissolve uric acid in the organism.

In a case of leukemia which showed increased uric acid excretion and urate deposits in urine, Bohland (1894) found that during piperazin treatment, as well as with potassium bicarbonate, and when no urate solvent was administered, the excretion remained the same. In spite of large doses, piperazin did not affect the deposits, but these were removed by potassium bicarbonate. Ebstein and Sprague (1891) administered 14 grams of piperazin within seven consecutive days and carefully observed the quantity of uric acid excreted together with proper control of dietary and fluid intake. There was only a slight, and practically negligible, increase in uric acid excretion, the urine output during this time being variable. Fauvel (1908) found that small doses of piperazin (1 gm. daily) diminished the excretion of endogenous uric acid, the diet during this time being purin free, but when the dosage was increased (2 to 4 gm. daily) the excretion of uric acid was increased, but not above the quantity before piperazin was ingested.

Ortner (1908) claims that piperazin seems in some cases to be effective as a therapeutic agent, its action being explained by its power to dissolve uric acid. However, Ortner emphasizes that he never prescribes piperazin alone, but with a bottle of Seltzer, Preblau or soda water, to which, no doubt, may be ascribed a considerable, if not the greater, share of the supposed solvent action. Using the colorimetric method of Folin, Robertson (1914) observed that piperazin as well as a number of other substances (pituitary, saline, $BaCl_2$, caffeine and Na_2SO_4) produced in hens no changes in uric acid excretion sufficiently independent of urine flow to suggest any specific effect of the agents used.

The following is a summary of reports favorable to piperazin, though scientifically not acceptable:

Abl (1913) claims that piperazin produced an increase in the urinary excretion of uric acid, and the same is reported by Abl for a number of diverse agents such as mustard, arsenic, colchicine, thorum X, sulphur, choline, chloral hydrate, neurine and strontium! The mechanism of action, Abl believes, is located in the intestine. However, it seems quite probable that diuresis at least in part (contrary to the claim of the author) and other factors, such as disease, destruction, circulatory changes, etc., were responsible for the results reported by Abl. Attaix (1896) made the claim that the quantity of uric acid and urates is augmented proportionately to the urea. This and other claims of this author are not supported by any discoverable scientific data. The effect of piperazidin on the excretion of uric acid was studied by Bardet (1891), but with no urinary or dietary controls, rendering it impossible to draw any conclusion from the work. Goodbody (1896) administered piperazin in 1 gm. daily doses for nine days, and 2 gm. daily during the next five days, maintaining the diet and fluid intake constant. Under these conditions uric acid excretion increased from 0.31 to 0.34 gm. on the 1 gm. dosage, and eventually increased to 0.37 gm. with the 2 gm. dosage. Goodbody concluded that administration of piperazin increases the elimination of uric acid, not by increased formation, but by rendering the blood more capable of removing it from the tissues by increasing its solvent power. However, it must be urged that these differences are small and within ordinary variations, as indicated by Goodbody's own results before the administration of the drug. Finally, granted that piperazin produced this increase in the excretion of uric acid, it is so small and the dosage so large, and the duration of administration so long that it would be practically valueless, uneconomical and undesirable.

7. EFFECT OF DIURESIS

There are many statements in the literature to the effect that piperazin can favorably influence diuresis, but the scientific evidences for this are deficient. Attaix (1896) makes the statement that piperazin lessens the density of urine and acts as a powerful diuretic. However, in view of the fact that only imperfect data are cited in connection with a single observation of his own, Attaix's conclusion is unjustified. The more careful observations of Bohland (1894) showed that piperazin did not change the quantity of urine voided in twenty-four hours. Ebstein and Sprague (1891) also found that while the urine output during administration of piperazin was variable, there was no marked change from diuresis before piperazin. Fraenkel (1912) referring to the general group of urate solvents, including piperazin, states that these agents possess chiefly a diuretic action, and dilution of urine facilitates excretion of uric acid. This statement, however, is unsupported by any data. Goodbody (1896) administered piperazin (1 to 2 gm. daily) for several days (fourteen) to an individual whose urine exhibited deposits of uric acid gravel regularly (using proper dietary and fluid intake controls) and found that 1 gm. of piperazin increased the volume of urine from 1,103 c.c. to 1,476 c.c., and 2 gm. caused a further increase to 1,680 c.c. A closer inspection of Goodbody's tables reveals the fact that diuresis before administration of the drug was variable, and on certain days before treatment with piperazin the values are as great as those during piperazin periods. It is hardly permissible, therefore, to ascribe any great importance to these results, particularly since the loss of water by other channels (sweat and the intestine) have not been considered. Heubach (1891-92) administered 2.5, 1 and 2 gm. piperazin on the first, second and third days, respectively, to two different individuals and observed that only a slight increase in urine output occurred with no diminution in specific gravity. Kobert (1897) states that all of the following are diuretic in doses of 1 to 2 gm. per day: piperazin, lycetol, lysidin and hexamethylenamin,

and that they may act in part by removal of water; but the evidence for this is lacking. D. D. Stewart (1893) administered 18 to 30 grains (1.1 to 2 gm.) daily of piperazin over long periods of time (fourteen days) consecutively in three cases of renal stone and observed an increase in urine volume in one case which could be explained by a hydronephrosis present that disappeared following an operation for removal of the stone. In a later communication, D. D. Stewart (1894) claims that two different preparations of piperazin used had invariably a diuretic influence. The elimination of urea and chlorid at the same time remained unaltered. Wittzack (1893) also claims that piperazin (using about 10 gm. per day) caused an increase in diuresis. However, it is not clear whether the fluid intake was controlled. Umpfenbach (1891) is reported to have observed a favorable influence on the quantity of urine excreted, and on the nerves and muscle of the bladder.

8. EFFECT ON THE REACTION OF URINE

The scientific data on this are conflicting, although most clinicians seem to be of the opinion that the reaction is unchanged. Aside from the observations of Haskins (1916) there are no data in the literature, so far as I know, regarding the true reaction (i. e. hydrogen-ion concentration) of urine as influenced by piperazin. Haskins found that the direct addition of piperazin or lysidin to acid urines lowers the hydrogen-ion concentration (from 5 to 7.4 with a piperazin concentration of 0.68 per cent.), i. e., the reaction was alkaline. However, after administration, the reaction of urine was uninfluenced and frequently remained acid. The reactions referred to in the following papers are presumably in many cases intended to convey changes with litmus, in others, by titration. Owing to these uncertainties, the value of the abstracts is considerably diminished. Nevertheless, it seemed worth while to refer to them.

Biesenthal (1892), citing the advantages of piperazin over ordinary alkalies and waters, states that after prolonged administration of piperazin the urine re-

mains acid, but he gives no scientific data. Bohland (1894) claims that the administration of piperazin in a case of leukemia slightly lowered the acidity of urine. Goodbody (1896) made observations as follows: The addition of 0.1 gm. piperazin to 220 c.c. of urine from a patient suffering with uric acid gravel, and 0.2 gm. to the same volume of urine from another patient whose urine regularly deposited urate, increased the acidity from 0.41 to 0.44 per cent. (NaOH) in twenty-four hours and 0.39 to 0.40 per cent. in thirty-six hours, respectively. In five cases the acidity of the urine was reduced from an average of 0.39 to 0.38 per cent. by the administration of 0.2 gm. piperazin. These differences are so small as to be practically negligible. Further observations by Goodbody on the effect of prolonged administration of piperazin in doses ranging from 1 to 2 gm. per day gave the following results: The average during normal days was 0.46 per cent. NaOH, and this fell to 0.36 per cent. on 1 gm. piperazin daily; 0.2 gm. piperazin daily caused a further fall to 0.30 per cent. The conclusion reached was that piperazin has the power to diminish the acidity of urine. It must be pointed out, however, that this does not necessarily mean there was a reduction in the true reaction of the urine. Fauvel (1908) found that the reaction of urine from individuals on a purin free diet was unchanged by small (1 gm.) or larger (2 to 4 gm.) daily dose of piperazin. The administration of 2.5, 1 and 2 gm. of the drug on the first, second and third days, respectively, by Heubach (1891-92) had no influence on the reaction of the urine, which remained acid. In the experience of Mordhorst (1892) piperazin causes only a small reduction in urinary acidity. In his observations, D. D. Stewart (1893-94) found that the acidity of urine was not appreciably affected by the administration of piperazin. On the other hand, Wittzack (1893) claims the acidity of urine is lowered, the urine, however, never becoming neutral or alkaline. It is not clear whether the influence of dietary in Wittzack's work was excluded or not.

EFFECT OF THE ADMINISTRATION OF PIPERAZIN ON CALCULI

This feature of piperazin therapy has undoubtedly been exaggerated. The statements and opinions held regarding the favorable influence that piperazin treatment is alleged to possess on the removal and solution of calculi are unsupported by a single iota of scientific evidence. For instance, the claim of Biesenthal (1892) for the superiority of piperazin over alkaline mineral waters as a preventive of stone formation is made without foundation of fact. He also makes the surprising claim that alkaline mineral waters lead to stone formation because of phosphate precipitation. Cushny (1910) observes that piperazin has not been shown to be of any value in the treatment of calculus. Referring to its use as a preventive of the formation of renal and vesical calculi and other conditions, Hare (1905) states that he has failed to obtain any results from the use of this drug in practice. Fawcett (1894) made observations as follows: An individual was given 15 grains (about 1 gm.) piperazin daily for two weeks. The twenty-four hour specimens of urine (preserved with chloroform) were collected and allowed to drip over a stone for the next twenty-four hours kept at body temperature. Two such experiments are reported. In one experiment the stone weighed 2.4 gm. before the experiment and 2.404 gm. at the end of the first week during treatment. The same stone in the second experiment weighed 2.4435 gm. before and 2.6155 gm. at the end of the second week. The stone contained 78.5 per cent. uric acid by analysis. The same individual's urine was again collected for fourteen days, piperazin (1:1,000) added to it and a portion of the same stone used in the previous experiments was added to it and another portion to water containing 1:1,000 piperazin. No solvent action was observed by piperazinized urine, but complete solution took place in the water. Fawcett's simple though ingenious experiments demonstrate clearly the inefficiency of piperazinized urines, obtained either by administration, or by addition of the drug directly, to dissolve urate stone.

Maramaldi (1897) reports rather optimistically that piperazin has the power to give specific symptomatic relief in renal colic due to calculus, because, as he puts it, "the patient claimed to feel like a new-born man" after 1 gm. piperazin per day. Morphine and other measures had been previously used, however, without effect. No objective evidence is offered. From experiments on pigeons, in whom it is claimed experimental urate deposits were successfully prevented and removed by the administration of piperazin, Meisels (1893) declares piperazin can easily dissolve uric acid calculi in dilute solutions, being superior in this respect to sodium borate and phosphate, and that the results with the drug clinically are gratifying. No clinical data are furnished by Meisels. Beneficial symptomatic relief in individuals suffering with calculi is claimed by D. D. Stewart (1894), whose work with the urate solvents is more critical than that of his contemporaries. However, Stewart makes it plain that the beneficial action cannot be explained by uric acid excretion and prefers to leave the mechanism of action unexplained.

The observations of Mendelsohn (1892) cast doubt on the alleged solvent action of piperazin for calculi. Mendelsohn found that while piperazin in water dissolves uric acid calculi, no solution either of stones or uric acid takes place in piperazinized urine. He explains also that no better results can be hoped for from injections of solutions of piperazin into the bladder because they can be retained for two or three hours only, and the solvent effect would decrease with the advent of urine, rendering the solvent power of piperazin practically useless, all of which clearly emphasizes the fact that when piperazin is used under conditions of the body no beneficial effect or solvent action of the drug is demonstrable. Mordhorst (1896), whose work has been cited in another place, also clearly explained why no beneficial therapeutic action may be expected from the so-called urate solvents. Penzoldt (1900) concurs in this view, also Poullsson

(1912). H. C. Wood (1902) is of the opinion that while piperazin may be of service as a solvent of uric acid gravel, it will not dissolve a ready formed calculus in the bladder.

10. EFFECT OF PIPERAZIN IN GOUT

The uncertain rôle that uric acid plays in the etiology and symptomatology of gout has not prevented certain clinicians from frequently recommending the so-called urate solvents as beneficial therapeutic agents in this condition. In view of the etiologic uncertainty, the use of such agents is certainly irrational. That the value of so-called urate solvents in gout may be considered negligible is amply confirmed by the reports in the literature which are conspicuous for lack of scientific evidence; judgment being further impaired because of the influences of other attending circumstances, various unknown factors, and allied medication. The following abstracts, therefore, possess only the value that may be attached to opinion unsupported by scientific evidence.

Heinz (1907) states that piperazin has been administered much in gout and "uric acid diathesis" without promising results. Penzoldt (1900) agrees with this. Because, as Kobert (1897) supposes, the so-called urate solvents act as diuretics, it is suggested that they act in gout by removal of water. However, Kobert's supposition regarding diuresis is not borne out by the facts. D. D. Stewart (1894) thinks that piperazin gives relief in gouty attacks. He is not clear as to how this is brought about, but he agrees that it certainly cannot be explained by any influence on the excretion of uric acid. Zimmermann (1901) reports gout in a parrot as having been permanently benefited by the administration of piperazin. However, the bird also received sodium bicarbonate internally, and salicylated collodion locally, rendering it, therefore, rather difficult to ascribe the beneficial effects observed to any one particular agent. Attaix (1896) reports a case of gout, which had previously been treated with colchicum and salicylate, but without response. The attacks, it is claimed, were prevented by piperazin. Biesenthal

and Schmidt (1892) cite five cases reported by Bardet who is said to have demonstrated an increase of uric acid excretion in gout, but neither the data nor the conditions under which these were obtained is mentioned.

In contrast to all this may be cited the paper of Fawcett (1894) as the only piece of scientific evidence available. Using the Hopkins quantitative method for uric acid, and suitable controls, Fawcett found that piperazin exerted no effect on the excretion of this metabolite in gout, or on the relief of the symptoms. He concluded that piperazin is not as valuable as certain drugs already recognized for the treatment of this condition.

11. UNDESIRABLE ACTIONS OF PIPERAZIN

Several reports in the literature indicate that the administration of piperazin may be attended with or followed by albuminuria, urticaria and other undesirable objective and subjective symptoms. Albuminuria due to piperazin has been claimed by Rörig (1893). Rörig reported repeated albuminuria in an individual who had had nephritis, but did not show albuminuria before the administration of the drug. Slaughter (1896) reports symptoms of collapse in an individual following the administration of 20 grains in a pint of water. Biesenthal (1891, 1893), however, claims that large doses of 6 gm. cause no disturbance of renal function. In criticising the report of Rörig, Biesenthal states that the picric acid reagent for albumin also gives a precipitate with piperazin which appears in urine after administration, and therefore is not characteristic. Bradford (1892) observed urticaria in a patient with gouty tendency following the ingestion of about 1 gm. of piperazin, but inasmuch as the individual complained of digestive disturbance and had also received phenacetin, salol and local applications it is apparent that no definite conclusions regarding piperazin alone can be made. Headache and some vomiting after doses of 2.5 gm. were experienced by Heubach (1891). In phlorizinized dogs piperazin

completely inhibits the glycosuria, and after fourteen days' treatment of a diabetic patient with 1 to 1.5 gm. doses (total 3), the sugar excretion fell from 8 to 3.3 per cent. (Hildebrandt, 1893). The activity of hydrolitic ferments was also found to be checked. Gruber (1893) reports similar results. Rabbits tolerate 0.5 to 1 gm. hypodermically according to Van der Klip (1892). D. D. Stewart (1894) states that following the administration of 70 grains of the drug per day there were intermittent, clonic spasms of extremities, muscular prostration, incoherence, incoordination, tremors, and these symptoms disappeared thirty hours after onset. Tremor, malaise and nausea were observed with smaller doses. According to Wittzack (1893) subcutaneous injections of piperazin are painful. Wood (1902) claims to have observed muscular weakness and general depression following continuous exhibition of large doses. Therapeutic doses in man, however, produced no symptoms. Van der Klip (1902) observed that sufficiently large doses of piperazin cause vomiting, irregular breathing, general muscular weakness and relaxation. The dissociation of oxygen from hemoglobin was lessened in a concentration of 1:5,000; a 0.5 per cent. solution favored the coagulation of blood and the action of peptonizing ferments was checked. Bohland (1894) also claims piperazin lessens the oxygen liberating property of hemoglobin because the spectroscopic band of oxyhemoglobin remained unchanged for days and weeks. According to Bohland piperazin is also an antiseptic and causes hemolysis. On the other hand, Cushny (1910) states that piperazin seems to induce no symptoms in man or animals even when used in large quantities.

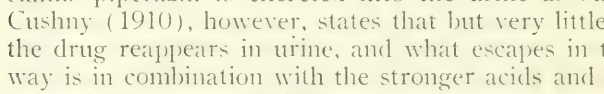
12. FATE AND EXCRETION OF PIPERAZIN

This is of importance in connection with the therapeutic use of the drug as a urate solvent, for if piperazin is largely destroyed in its passage through the body, much could not be expected of its supposed solvent action in urine. According to Mordhorst (1898) excretion of the drug itself occurs in urine,

but so much is destroyed that a considerable reduction of the concentration occurs in the body. Van der Klip (1892) detected the drug in four hours in rabbit's urine. Neubauer and Vogel (1898) state that the greater part of the drug is excreted in a few hours; the excretion lasts a few days, and the drug appears unchanged in the urine. According to Wood (1902) piperazin is rapidly absorbed and eliminated by the kidneys, producing a reddish-brown urine. With the aid of the bismuth-iodid reagent, Helbing and Passmore (1894) claim to have determined that the greater part (11 grains) of 15 grains of piperazin was excreted unchanged. By means of the same reagent Zimmermann (1901) demonstrated the presence of piperazin in the cloacal contents of a parrot. Biesenthal (1893) claims piperazin is excreted into the urine as such. Cushny (1910), however, states that but very little of the drug reappears in urine, and what escapes in this way is in combination with the stronger acids and not uric acid. Fraenkel (1906) states that piperazin passes unchanged in urine, and is excreted rather rapidly, oxidation also taking place. The opinion in general seems to be that piperazin is decomposed to a certain extent and bound with acids in the body, and in this way robbed of any activity it may possess (Poullsson, 1912; Heinz, 1907).

13. OTHER URATE SOLVENTS

Lycetol.—Chemically this is dimethyl piperazin tartarate. Its structural formula is



The behavior of lycetol in the body differs in no particular from piperazin (Fraenkel, 1906). Anthony (1895) claimed beneficial results in six cases of gravel. However, in the absence of any data whatsoever, this report may be regarded as a mere testimonial. Basile (1901) observed that lycetol can dissolve added uric

acid, but that it is variable, the differences being due to variable qualities of urine. Added to urine in the concentration of 1 per cent. lycetol dissolved about 0.7 per cent uric acid, whereas after administration, the excreted portion in urine dissolved only about 0.4 per cent. Lycetol, therefore, loses considerable uric acid solvent power by passing through the body. So far as urate calculi are concerned, 1 per cent. lycetol dissolved about 0.15 to 0.25 per cent. of uric acid, but had only a weak effect on mixed calculi. Basile also studied the effects of the administration of lycetol to twenty-six patients and concluded as follows: Diuresis was augmented (from 910 to 1,100 c.c.); gravel disappeared from urine; the quantity of uric acid excreted remained small or uninfluenced in the beginning, but later was augmented; clinically the patients who suffered with "uric acid diathesis" became improved; the specific gravity of urine was not appreciably reduced; urinary acidity was diminished, some urines becoming neutral, others alkaline. Beneficial results in gout and sciatica are also claimed by Basile. While the relief in the various conditions was attributed by Basile chiefly to the urate solvent power of lycetol, this can hardly be the case altogether, because of the small and uncertain uric acid values reported by him, the uncertainty of factors influencing this, and because of the possibility of spontaneous relief in these conditions without drugs. In much the same way may be regarded the symptomatic relief in gout and sciatica reported by De Tollénaere (1897).

Hoven (1898) reported beneficial results with 1 gm. of the drug in gouty individuals, and attributed this favorable action to increased alkalinity of the blood and urine, but these conclusions are not justified for lycetol alone because of the simultaneous use of other medication, such as magnesia usta and liberal quantities of mineral water. Eight and nine-tenths per cent. of added uric acid was dissolved by lycetol at room temperature as observed by Meisels (1902). However, the nature of the solvent (urine or water) is not stated in the only abstract available. Lycetol has no

influence on the excretion of uric acid by birds with experimental gout (Wauchomont, 1912). The clinical condition was also uninfluenced.

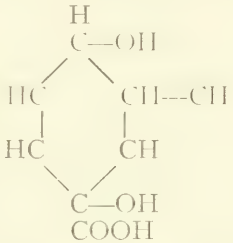
Sidonal.—This is a piperazin salt of quinic acid, called piperazin quinate and possesses the following chemical formula:

$$NH\begin{matrix}/CH_2CH_2\backslash \\ \backslash CH_2CH_2/\end{matrix}NH.2C_6H_7(OH)_4.COOH.$$

The effects on uric acid or urate should differ in no essential respect from the effects of the individual components, and this seems to be the case according to the reports in the literature. Bardet (1901) administered 3 to 5 gm. of the drug daily to three patients and observed its effect on diuresis, uric acid excretion and reaction of urine. It was found that these remained practically uninfluenced. According to Blumenthal (1900) the excretion of uric acid is reduced by 40 to 50 per cent. with daily injections of 5 to 8 gm. of the drug, and in place of uric acid, hippuric acid appears in the urine. Mylius (1900) makes a favorable report, unaccompanied by any data, on the use of sidonal in gout. Rosenthal (1900) reports that in a case of gout the administration of 100 gm. of sidonal led to the reduction of uric acid in urine from 0.75 gm. per liter to 0.332 gm., and that the tension of the skin over the tophi and redness disappeared. After a pause of three weeks, 100 gm. of sidonal was administered with similar improvement. This was repeated again in three months, and six months later the condition is said to have remained permanently improved. Klemperer (1900) is dubious about the therapeutic value of sidonal in gout, because the value of piperazin has not been demonstrated and, in his experience, quinic acid is worthless. The experiments of Foerster (1899) showed that the administration of sidonal (to six patients) causes no important differences in the excretion of uric acid. Ten to 25 gm. of the drug were used daily for three to five days, using suitable controls. Foerster could not confirm the reported claims of his day, and pointed out that some other explanation for

the reported effects must be sought rather than the drug itself. Meisels (1902) reports that sidonal dissolves 35.5 per cent. of added uric acid, but the temperature and solvent used are not stated in the only report available. Schlayer (1900) reports such beneficial effects as reduction in uric acid excretion from the administration of sidonal to six cases of "uric acid diathesis," increased diuresis, diminished turbidity of urine and lessening of renal pains in an individual with renal stone. Waucomont (1912) observed no beneficial effects on the clinical condition of experimental gout in birds.

Quinic Acid.—This is closely related to benzoic and other aromatic acids. Its formula is



It has been recommended as a urate solvent, but its mode of action has remained unexplained. It has recently been shown by Denis (1915) that the administration of quinic acid has little or no effect on the uric acid content of urine or blood, leaving the threshold of the kidney for uric acid unaffected, and differing in this respect from salicylate and atophan. Likewise the information obtained from the literature referred to in this section indicates that quinic acid possesses no special virtues as a urate solvent.

According to Cushny (1910) quinic acid has no effect whatever on the quantity of uric acid excreted. Foerster (1900) claimed to have demonstrated an increase in uric acid excretion and urine output following the administration of a total of 50 gm. in several days. Unfortunately the results are complicated by the simultaneous administration of thymus gland. Fraenkel (1912) states that Weiss observed

a diminution in uric acid excretion. Hupfer (1903), however, denies the lessening effect on uric acid excretion by quinic acid. The experiments of Meisels (1902) on pigeons with artificial urate deposits seem to indicate that quinic acid is less efficient than sidonal as an inhibitor of the deposits. In human individuals sidonal increased the excretion of uric acid from 0.3289 to 1.968 gm. Taltavall and Gies (1903) found that in dogs on constant diets, quinic acid (1 to 20 gm. in 10 days) did not affect the elimination of uric acid. This agrees with the observations of Hupfer on human individuals. This was further confirmed by Ulrici (1901), who also showed that quinic acid does not influence nitrogenous metabolism.

Lysidin.—Chemically this is ethylen-ethylen diamine, and the formula is

$$\begin{array}{l} CH_2 \cdot \quad N \diagdown \\ | \qquad\quad C \cdot CH_3 \\ CH_2 \cdot NH \diagup \end{array}$$

Lysidin is said to possess five times the solvent action of piperazin (Fraenkel, 1912). According to Goodbody (1896), urine containing 0.1 per cent. of the drug increased the solution of urate in normal urine from 0.059 to 0.065 per cent.; the acidity was diminished from 0.35 to 0.33 per cent. Following the administration of 1 gm. of lysidin daily for eight days, and 2 gm. daily for four days, maintaining the diet, fluid intake, and exercise constant, Goodbody observed an increase in diuresis, increase in uric acid excretion, but no important changes in urinary reaction. In these particulars lysidin was found to excel piperazin. Haskins (1916) found that lysidin can act as a uric acid solvent (added uric acid) in acid urine. The urinary reaction may remain truly acid. If the urine is alkaline, when either lysidin or piperazin is administered, its uric acid solvent property is enhanced, but it is not a practical therapeutic agent because if large enough doses are used it is unsafe and possesses no advantage over sodium citrate or bicarbonate. Meisels (1902) observed that lysidin in aqueous solution can dissolve 47.86 per cent. of added uric acid. Meisels

also observed that the administration of lysidin gave relief to pigeons with urate deposits. According to Neubauer and Vogel, Ladenburg (1894) claims lysidin urate is soluble in 6 parts of water.

Clinically, however, Ortner (1908) states he cannot recommend lysidin as a urate solvent, adding that "it not only has never done any good in his experience, but once, after a few days' use, it caused an almost general acute, painful, and partly bullous eczema." According to Schmiedeberg (1909) lysidin dissolves uric acid in vitro more easily than lithium carbonate, but the addition of 1 per cent. sodium chloride completely inhibits this. Favorable reports on the use of lysidin in gout and urate deposits are made by Wolf (1907). Lysidin is not regarded as an effective calculi solvent by Casper (1897). According to Ortowski (1900) lysidin is inferior to hexamethylenamine as a uric acid solvent. By the addition of uric acid to urine containing lysidin (0.5 per cent.), Tunicliffe and Rosenheim (1908) found the solvent power to be 33.2 when that of urine alone was expressed as 1. The solubility of sodium biurate in beef serum containing lysidin (0.1 per cent.) was 1 : 25,000 as compared with 1 : 60,000 in serum alone. The solvent power of piperidin was higher, 1 : 12,000.

Urol.—Chemically urol is a urea salt of quinic acid (2 molecules of urea combining with 1 molecule of quinic acid), and according to Frieser (1902) is beneficial in gout, rendering the urine free of "uric acid excess." However, no data are offered to substantiate this vague claim.

Urosin (lithium quinate).—The administration of 10 to 25 gm. daily for 3 to 5 days produced no important differences in the excretion of uric acid, nitrogen and phosphate (Foerster, 1900). Foerster could not confirm the reported claims of other observers, such, for instance, as that of Weiss (1900), who claimed that urosin lessens uric acid formation because of its quinic acid content.

Salicylate.—The chemical formula for salicylate acid is $C_6 H_4$ OH. COOH. It has been frequently

shown that the administration of salicylate increases the elimination of uric acid, but the mechanism of action has not been thoroughly understood. It has recently been shown by Denis (1915) that the administration of salicylate leads to a diminution of uric acid in the blood with a simultaneous increase in the uric acid content of the urine, and this is interpreted as due to a lowering of the threshold of the kidney for uric acid excretion. The earlier literature has been abstracted in a previous contribution (Hanzlik, 1915) and need not be further detailed here.

Phenyl-Quinoline.—The effects of a number of phenyl-quinolines on uric acid excretion were studied by Luzzatto and Ciusa (1913) with negative results. The most favorable results were obtained with diphenyl-quinoline carboxylic acid (atophan) and diapurin (a-phenyl-b-naphthoquinoline carboxylic acid)

It would be altogether out of place here to refer to the rather extensive literature on atophan. Other sources (Sollmann, 1917; Fraenkel, 1912) may be consulted for this. Briefly, however, it may be stated that atophan is related chemically and pharmacologically to salicylic acid. For lack of any other explanation it was generally held that atophan is a "mobilizer" of uric acid in the tissues. However, it has been shown by Folin and Lyman (1913) and Fine and Chace (1914) that the effects of atophan on the excretion of uric acid consists of an increased elimination in the urine with simultaneous decrease in the uric acid content of the blood. Haskins (1913) also observed that atophan increases the elimination of uric acid in the urine.

Colchicum.—This was found by Rockwood and Van Epps (1900) and Abl (1913) to decrease uric acid excretion. On the other hand, an increase in excretion was reported by Jackson and Blackfan (1907). Most recently, however, it has been shown by Denis (1915) that the administration of wine of colchicum exerts little or no influence on the uric acid content of the blood and urine.

Piperidin.[2]—Tunicliffe (1897) found 50 c.c. of a 10 per cent. solution to completely dissolve 10 gm. of uric acid. The piperidin urate could be completely precipitated by sodium chloride and its solubility was found to be 5.3 and 25 per cent. at 17° and 36°, respectively. Aqueous solutions possessed an alkaline reaction. According to Tunicliffe his results do not confirm the high solvent power ascribed to piperidin urate by others.

14. SUMMARY

There is no reliable evidence to show that piperazin, in small or therapeutic doses, imparts to urine urate solvent qualities, either by direct addition or after excretion.

Excessive doses of the drug produce a slight but practically negligible increase in uric acid excretion, the same being effectively secured by the use of such well known alkalies as bicarbonate and citrate.

The solvent action of low concentrations of piperazin on calculi is practically negligible. In very high concentrations a solvent power, though limited and doubtful, seems to exist.

There is no reliable evidence to indicate that piperazin can prevent or remove urate deposits.

Diuresis is uninfluenced by the administration of even large doses of piperazin.

The direct addition of piperazin to urine renders it alkaline. However, after administration the reaction remains unchanged because in its passage though the body enough piperazin is destroyed to markedly reduce its concentration in the urine.

Scientific evidence, though limited, and clinical opinion indicate that piperazin is valueless in gout.

The administration of piperazin may be attended with serious side actions.

There is sufficient scientific evidence to indicate the worthlessness of the following as urate solvents: urosin, lycetol, sidonal, quinic acid, lysidin, urol, quinoline, colchicum and piperidin.

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2. For description see footnote, Section 4.

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SOME EXPERIMENTS ON THE CHEMICAL REACTIONS OF DIPHTHERIA ANTITOXIN *

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AND

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(*From the American Journal of Pharmacy, April, 1917*)

Many of us expect to find that future advances in rational therapeutics will be made along chemotherapeutic lines and by following the methods used by nature, i. e., by the use of antitoxins, etc. Hence it is essential to know something as to the chemistry of the antitoxins. From this point of view, we recently reviewed the literature on the chemistry of diphtheria antitoxin.[1] As a result of this summary, it becomes evident that there are two views; one is, that the antitoxin is not necessarily a globulin, but is carried down with them on precipitation; the other view, held by most workers, is that diphtheria antitoxin is a globulin, and some uncorroborated work even suggests that ordinary egg globulin could be converted into diphtheria antitoxin.

We have been carrying on experiments to determine the reaction of diphtheria antitoxin to various reagents, and assuming it were not a globulin, to find whether it could be separated from the globulins. No doubt the response to reagents will vary, according to the solution in which the antitoxin occurs.

For part of this work we have used unconcentrated preparations obtained through the courtesy of the Cutter Laboratory at Berkeley. The first preparation contained over 500 antitoxic units to 1 c.c. It was

* The expense of this work was partly covered by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Crawford, A. C., and Foster, M. G.: Biochem. Bull., 1917, **6**, 1.

prepared by heating a mixture of 70 parts of diluted antitoxic serum with 30 parts of saturated ammonium sulphate solution, i. e., Banzhaf's method. The second preparation was simply a solution of the precipitate from antitoxic serum by from 30 to 50 per cent. saturation of ammonium sulphate. It contained 450 units to 1 c.c. The third preparation was a concentrated globulin solution (40,000 units in about 13.5 c.c.). This was prepared by a modified Banzhaf method and was given us by Parke Davis & Co. The fourth was a globulin preparation from the Cutter Laboratory and contained 250 units per 1 c.c.

The determinations of the antitoxic values were made by the firms from which the preparations were obtained and controlled, when any variation was suspected. The toxin was made by the Cutter Laboratory and its L + dose determined there, but was checked with standard antitoxin obtained from Dr. G. McCoy of the Hygienic Laboratory.

In these experiments usually only three guinea pigs were used for each test. The injections were made subcutaneously in the midabdominal region and each injection had a volume of 4.5 c.c.[2] All evaporations were done in vacuo between 50 and 55 C., and preparations which were not used immediately were usually kept in an ice box. Instead of filtering, solutions were obtained by centrifugalizing at 4,000 revolutions a minute.

The guinea-pigs weighed about 250 gm., although owing to difficulty in obtaining a proper supply, guinea-pigs below 250 gm. were often used At the autopsy of those dying after the injections, enlargement and hemorrhages into the suprarenal glands, hemorrhages in the gastric mucosa and signs of local irritation were the only macroscopic changes looked for and found. No histologic examinations were made.

Some of globulin preparation IV was evaporated to dryness in vacuo, and the flask jarred with a vibrator for varying intervals of time from July 20 to July 31,

2. Rosenau, M. J.: Hyg. Lab. Bull. 21, 1905.

1914, then put aside till February, 1917, but no signs of crystallization have appeared.

Fifteen c.c. of globulin II were shaken with 400 mg. cholesterin and centrifugalized. The fluid retained its full antitoxic value.

After precipitation of antitoxin with aluminium hydroxide[3] the filtrate of globulin preparation IV became inactive.

Five c.c. of globulin preparation I were evaporated to dryness and then twice extracted at room temperature with 20 c.c. acetic ether c.p. The undissolved portion possessed about the antitoxic value of the original, showing the antitoxin to be insoluble in acetic ether.

Five c.c. of globulin preparation I were evaporated to dryness then extracted with 20 c.c. *n*/10 NaOH and after a few minutes neutralized with 20 c.c. *n*/10 HCl. Even three times the theoretical amount which should neutralize one L + dose failed to protect. Evidently *n*/10 NaOH injures this antitoxin.

The same amount of this globulin was evaporated and treated with 20 c.c. of *n*/100 NaOH, and after ten minutes was neutralized with 20 c.c. of HCl *n*/100. This solution retained the full antitoxic value of the original, i. e., short contact with *n*/100 NaOH does not injure antitoxin. No attempt was made to find if prolonged contact would injure it.

Five c.c. of globulin I were treated with 5 c.c. NaOH *n*/10, then neutralized with 5 c.c. HCl *n*/10. This was diluted to 150 c.c. Two c.c. of this solution protected against one L + dose of the toxin. Apparently there was some slight loss in activity.

Five c.c. globulin II was diluted with 5 c.c. NaCl (0.85 per cent.), treated with 10 c.c. NaOH *n*/10 and neutralized with 10 c.c. HCl *n*/10. This had about the full antitoxic value, i. e., *n*/20 NaOH does not injure the antitoxin, at least in this preparation.

Ten c.c. globulin preparation II were treated with 10 c.c. NaOH *n*/10 and shaken with benzol. The benzol residue was shaken with 25 c.c. NaCl (0.85 per

3. Jour. Am. Chem. Soc., 1913, p. 820.

cent.). Even 3 c.c. did not protect against one L + dose of the toxin. The mother fluid contained the full antitoxic value, showing that *n*/20 NaOH did not injure this antitoxin, and that it would not shake into alkaline benzol.

Ten c.c. of globulin II were treated with 5 c.c NaOH *n* 10 and shaken with ether. The ether residue contained no antitoxin, while the mother fluid had its full value, i. e., antitoxin is not soluble in alkaline ether.

Five c.c. of globulin preparation I were evaporated to dryness in vacuo and extracted twice with 20 c.c. of methyl alcohol (Merck). This alcohol was evaporated and the residue left by its evaporation was extracted with 10 c.c. normal NaCl. Even 3 c.c. of this did not protect from one L + dose of the toxin. Presumably no antitoxin was present.

The residue after methyl extraction was dissolved with 20 c.c. of NaCl (0.85 per cent.) and 10 c.c. *n*/10 NaOH. It did not dissolve in NaCl. It was then neutralized with 10 c.c. *n*/10 HCl. This solution had the full antitoxic value of the original solution. The diphtheria antitoxin as present in this preparation is insoluble in methyl alcohol.

The same amount of preparation II was evaporated to dryness and treated with methyl alcohol containing the same amount of NaOH as used in the above test, but as very small amounts seemed to be dissolved, no tests were made on animals.

Five c.c. of preparation II were treated with 5 c.c. of *n*/10 NaOH and 40 c.c. methyl alcohol (Merck), and after standing about twenty to thirty minutes were centrifugalized. The precipitate was dissolved in 25 c.c. *n*/100 NaOH and then neutralized with the same amount of *n*/100 HCl. This gave a solution much the color of the original globulin preparation, although the precipitate was only slightly colored. This solution was slightly less active than the original preparation, perhaps due to standing, as several days elapsed before we were able to test this preparation.

Ten c.c. of globulin preparation I were diluted with 10 c.c. of NaCl (0.85 per cent.) and precipitated with

a solution of lead subacetate, drop by drop, from a burette. The preparation was then centrifugalized and the supernatant fluid precipitated with sodium hydrogen phosphate, centrifugalized and then diluted to 150 c.c. Even 3 c.c. did not protect against one L + dose of the toxin.

Several other attempts were made to free antitoxin from proteins by means of lead subacetate solution, but in most cases the filtrate when freed from lead was inactive. In one case it possessed slight antitoxic value, but in this case a possible excess of the alkaline lead solution may explain the result, i. e., solubility of antitoxin or globulin in weak alkali. In this latter case the lead filtrate contained a few antitoxic units, yet produced no anaphylactic reaction in a guinea-pig.

Ten c.c. of globulin preparation I were diluted with 10 c.c. of sodium chloride (0.85 per cent.) and precipitated with a cold saturated aqueous solution of picrolonic acid by means of a burette, then centrifugalized. The centrifugalized fluid was shaken with acetic ether to remove picrolonic acid, at least partly, although acetic ether did not seem to us as suitable for this purpose as isobutyl alcohol. After separating the undissolved acetic ether, the solution was diluted to an arbitrary amount (250 c.c.). Even 3 c.c. of this solution failed to protect against one L+ dose of the toxin. The precipitate was shaken with NaCl (0.85 per cent.) and made into a colloidal suspension of 250 c.c. Only 1 to 3 c.c. were tested. One c.c. of this suspension protected against one L + dose of the toxin, showing that most and perhaps all of the antitoxin was in the picrolonic acid precipitate.

A similar preparation was also precipitated with picrolonic acid. The precipitate was shaken into a colloidal solution or suspension with distilled water. This was centrifugalized and the sediment shaken with NaCl (0.85 per cent.). The H_2O solution was diluted to 250 c.c. One to 3 c.c. were tested. One c.c. protected against one L + dose. Presumably the neutralizing power was even greater than shown. The colloidal solution obtained with 0.85 per cent. NaCl

was diluted to 350 c.c. Even 1 c.c. protected against one L + dose of the toxin. Some antitoxin went into both preparations.

Three c.c. of globulin preparation III were precipitated over night with a saturated aqueous solution of picrolonic acid. After centrifugalizing the clear solution was shaken several times with iso-butyl alcohol. The alcohol gave no precipitate. The colorless solution was made up to 250 c.c. Even 3 c.c. of this did not protect against one L + dose of the toxin. Evidently the filtrate, i. e., the centrifugalized solution after picrolonic acid contained no antitoxic units. This highly concentrated globulin corresponded in its reaction to picrolonic acid to preparation I which was of a lesser concentration.

The precipitate from picrolonic acid was shaken with NaCl (0.85 per cent.) and made into a suspension. This was shaken several times with iso-butyl alcohol to remove picrolonic acid. The iso-butyl alcohol precipitated a gelatinous mass, which after centrifugalizing became colorless on further shaking with iso-butyl alcohol. This white gelatinous material was dissolved in NaCl (0.85 per cent.) by the addition of NaOH $n/10$ and the corresponding amount of $n/10$ HCl was then added. This colloidal solution was made up to 500 c.c. with NaCl (0.85 per cent.). This solution had antitoxic value, but did not correspond to the full number of units used. This may perhaps have been due to the long contact with iso-butyl alcohol.

The centrifugalized solution after iso-butyl alcohol precipitation was made up to 100 c.c., but even 3 c.c. did not protect against one L+ dose, so that it contained few if any antitoxic units. Evidently iso-butyl alcohol precipitates antitoxin at least from this preparation.

Two c.c. of preparation III (8,000 antitoxic units) were precipitated with a saturated aqueous solution of picrolonic acid and the precipitate was shaken several times with NaCl (0.85 per cent.) centrifugalized, then the precipitate shaken again, then filtered through filter paper. The filtrate was then made up to 750 c.c. Even 3 c.c. did not protect from one L + dose of the

toxin. The loss of activity may have been due to the filtering through filter paper, or to the preparation having stood several days, but it was thought, as there was picrolonic acid present, that this should preserve it.

Ten c.c. globulin preparation I, diluted with an equal volume of NaCl (0.85 per cent.), was precipitated with a saturated aqueous solution of uranium acetate c. p., then centrifugalized. The precipitate was dissolved in normal salt solution by the addition of a few drops of *n*/10 NaOH. The uranium was precipitated by Na_2HPO_4 and centrifugalized. This solution was diluted to 250 c.c., an arbitrary amount. It was found that one c.c. protected from one L + dose. Less was not tried. This solution gave a precipitate with picrolonic acid, gold chloride, platinum chloride, copper acetate and alcohol. The filtrate after precipitation with Na_2HPO_4 and centrifugalizing was diluted to 250 c.c. Even 3 c.c. of this solution did not protect from one L + dose. Uranium acetate precipitates antitoxin.

Globulin preparation I, diluted with an equal volume of normal NaCl, was cautiously precipitated with uranium acetate solution, then centrifugalized. The precipitate was treated with 15 c.c. NaCl (0.85 per cent.) and 15.6 c.c. *n*/10 NaOH. This formed an emulsion. The emulsion was precipitated with Na_2HPO_4, then precipitated with picrolonic acid. The precipitate was suspended in NaCl (0.85 per cent.) and shaken with iso-butyl alcohol (Merck) to remove the picrolonic acid. The solution was diluted to 250 c.c. Even 3 c.c. did not protect from one L + dose.

The iso-butyl alcohol gave a white precipitate which was made into a colloidal solution with 250 c.c. NaCl (0.85 per cent.). Two c.c. of this solution protected against one L+ dose of the toxin.

Five c.c. of preparation II (2,250 units) were precipitated with platinum chloride (10 per cent.) aqueous solution. The precipitate was shaken with *n*/100 NaOH as well as with NaCl (0.85 per cent.) centrifugalized, neutralized and diluted to 350 c.c. Even 3 c.c. of this solution did not protect against one L +

dose of the toxin. A second sample was likewise precipitated with the same platinum chloride solution and the precipitate stirred with distilled water, then with NaCl (0.85 per cent.) and then centrifugalized. The washings from the precipitate and the centrifugalized solution from the platinum were added together and warmed on a bath to 65 C.; then, while warm, were saturated with H_2S and the gas boiled off in vacuo. In this case the platinum sulphide separated nicely giving practically a colorless solution. There is some difficulty in obtaining a colorless solution in every test. The solution was filtered through filter paper and diluted to 100 c.c. One c.c. protected against one L + dose. After standing two days, 1 c.c. of this solution was diluted to 22 c.c. Three c.c. of this dilution did not protect against one L + dose of the toxin.

The 100 c.c. solution gave no precipitate with uranium acetate c. p., gold chloride, mercuric chloride, or one half saturation with ammonium sulphate, but gave a slight precipitate with picrolonic acid. Picric acid gave no precipitate. This concentration gave no biuret reaction and no test for tryptophane with magnesium glyoxalate and sulphuric acid.

To see if the last dilution was inactive owing to deterioration, no extra preservative having been added, the original 100 c.c. was tested 4 days after its preparation and 1 c.c. still protected against one L + dose of the toxin.

A similar precipitation was made with a freshly prepared solution of platinum chloride (10 per cent.) and the precipitate was washed with NaCl solution instead of with distilled water as in the preceding case. The washings and centrifugalized solution could not be freed from platinum by H_2S alone, even on adding an excess of platinum, but cleared with H_2S when 1 c.c. of HCl $n/10$ was added to the solution (111 c.c.). After dilution to 150 c.c. it was found that even 2 c.c. did not protect against one L + dose of the toxin.

Five c.c. of the same antitoxin was precipitated with a solution of platinum chloride made two days previously. The precipitate settling on centrifugaliz-

ing was washed with distilled water as in the first experiment, and after passing H_2S became colorless save for a minute trace of golden color. This was diluted to 150 c.c. Even 1 c.c. protected against one L + dose of the toxin.

From these experiments it is evident that NaCl interferes with the precipitation of platinum unless acid is added.

There are several ways of interpreting the activity of the platinum filtrate; first, that it contained the antitoxin free from globulin or that the acidity, which resulted from passing H_2S, weakened the toxin, or that a trace of colloidal platinum sulphide remained in solution and weakened the toxin.

The acidity of the first platinum preparation corresponded to 0.3 c.c. $n/10$ HCl to each c.c. The second, in which much NaCl had been used and which was inactive, reacted for 0.55 c.c. $n/10$ HCl for each c.c. The third preparation which contained about as many antitoxic units as the first platinum preparation, reacted for 0.2 c.c. $n/10$ HCl to each c.c.

The L + dose of the toxin (0.42 c.c.) was treated with 0.42 $n/10$ HCl and let stand in the thermostat for one half hour, then neutralized with 0.42 $n/10$ NaOH. This preparation killed a guinea-pig in twenty-four hours, the same time as the untreated toxin. Evidently weakening of the toxin by acid was not the cause of the survival of the guinea-pigs after injection of the toxin mixed with platinum filtrate from the globulin preparation.

To see if an excess of acidity was the cause of the inactivity of the second test, 1 c.c. of preparation II was mixed with 1 c.c. HCl $n/10$ placed in an incubator for one half hour, then neutralized with NaOH. This was then diluted to 20 c.c., i. e., to theoretically correspond to the dilution in the second platinum experiment. One c.c. of this solution protected against one L + dose of the toxin, thus showing that this amount of acid did not destroy the antitoxin.

As a control test, 7.5 c.c. of platinum chloride (10 per cent.) were diluted to 100 c.c. with distilled water and while warm were saturated with H_2S. On filter-

ing this gave a solution perhaps darker in color than the preceding active platinum filtrate. This color was due to the presence of a trace of platinum sulphide. Injections were made of 1 c.c., 2 c.c. and 3 c.c., but these did not kill, or even sicken the guinea-pigs. Of this solution, 1 c.c. and 2 c.c. were each mixed with one L. + dose of the toxin diluted as usual, and placed in the incubator for one half hour. Even 1 c.c. protected against one L. + dose of the toxin, showing that the protection was due to the small amount of platinum present and that the antitoxin had not been freed. This action must presumably have been due to some catalytic action of the platinum as the concentration was presumably too weak to precipitate any of the toxin. These results may suggest a therapeutic use for platinum compounds.

From our review of the literature and from our own work at present we find no chemical proof that a separation of antitoxin and globulin can be made, although Banzhaf's work and that of Hurwitz and Meyer might suggest it.

Note.—Several of the guinea-pigs on which the platinum experiments were made developed abscesses.

SUGGESTED FORMULAS FOR PARAFFIN FILMS *

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The popular propaganda for "Ambrine" has brought the subject of paraffin-film treatment of burns into prominence. The results are said to be better than are obtained by other methods of treatment; however, neither "Ambrine" nor any other preparation could accomplish "miracles." The principle of the method is supposed to be mainly if not solely mechanical; the film of paraffin being impervious, forms a protection to the exposed tissues. On the other hand, it can readily be removed when desired. Perhaps the paraffin also forms a sort of scaffolding for the feeble granulations.

The method at least deserves scientific investigation. Such investigation, however, is hampered by the optimism which has developed in the minds of even medical men, regarding the efficacy of this treatment, resulting from the sensational accounts of the use of the secret French preparation, "Ambrine," in the present war. Another serious obstacle is the secrecy of the preparation so exploited, since it complicates any attempt at improvement. If the principle of paraffin films is a useful one, it is open to question that "Ambrine" is the *ne plus ultra* of these films. It is one of the disadvantages of the secrecy that we do not know what attempts have been made to secure the best possible preparation; and in the absence of this knowledge, it is reasonable to suppose that the preparation is capable

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of modifications which might be improvements. Perhaps extensive investigations have already been made in this direction; but of this we know nothing. If they have been made, we do not know whether or not all the possible lines of modification were taken into account.

The subject was brought to my attention by Dr. George W. Crile, and after weighing the foregoing considerations, it has seemed to me worth while to devise a series of paraffin combinations, so that the advantages of the various types of films could be tried out fairly. Since I started on this investigation, two American preparations have been placed on the market. Both have followed the French example of keeping the composition secret, and both therefore are open to the foregoing objections. This secrecy makes a systematic comparison of the different films difficult. However, I have compared the physical properties of these commercial preparations with the formulas which I have given herein. Since experimentation with preparations of unknown composition cannot add much of permanent value to our knowledge, and is apt to be largely wasted, it seems worth while to publish the formulas and properties of our preparations without awaiting their clinical trial. Indeed, the very object of this publication is that they may receive such clinical trial.

In planning the formulas for these waxes, I intentionally avoided a slavish imitation of the pretended composition of the proprietary preparations. On the contrary, I have aimed to make the formulas simple, each containing at most two constituents; to make the manipulations so simple that the preparations could be made independently, and if possible extemporaneously, by any pharmacist, and would thus be accessible to any surgeon who cared to try them; to vary these formulas so as to produce preparations with properties which differ considerably, and to devise simple methods for comparing the relevant physical properties. I have intentionally refrained from adding any deodorant, antiseptic, coloring matter,

etc. I believe that the first step should be to determine the suitability of these films from a purely mechanical standpoint. After the suitable type or types have been selected, it will be an easy matter to modify them by such additions.

The significant properties of the films appear to concern in the first place the melting point. This should be not much lower than 48 C., and not much higher than 53 C. Within this range, I incline to believe that the melting point is practically immaterial.

The hardness of the wax may be important. The harder the wax, the more firm is the support which it affords; but on the other hand, the softer films are probably more "soothing." It is quite possible that different cases may demand different types of films in this respect.

A further important property is the strength of the film (fragility). It involves at least two factors: (1) ductility, the coherence of the film against pulling and kneading—a property which reflects the resistance of a film against stretching; and (2) pliability, the resistance to fracture on bending.

The first of these (the resistance to pull) can be determined only roughly by comparative methods; there is no convenient quantitative measure of this, although one could perhaps be devised. The resistance to breaking can be determined quantitatively in relation to the temperature: the more fragile films will break on bending at a relatively high temperature, while the more plastic films can be bent at relatively low temperatures.[1]

General Methods of Preparation. — The mixtures were prepared simply by melting the ingredients in a water bath, after which the mixture was heated to 145 C. for sterilization.

Melting Point Determination.—This was attempted by the U. S. P. method, which consists in drawing the melted wax into a capillary tube; cooling for a certain

1. Further experience indicates that ductility may be expressed quantitatively by the temperature at which a thin film breaks sharply when pulled in a straight line.

period; attaching the tube to a thermometer; immersing in a water bath, and heating slowly until the melted wax begins to rise in the capillary tube.

The last clause of this method was not found very satisfactory with the more viscid fats: the slow heating gave a variable melting point usually several degrees higher than that obtained by immersing the capillary directly into the bath, which had previously been heated to the required temperature. The explanation probably lies in the viscidity of the oil when the column in the capillary tube is heated throughout, as it must be when the heat is raised gradually. Evidently the lowest melting point must be the correct one, so that our modification appears fully justified.

Determination of Hardness.—This was determined at 22 C. by trying the cakes of the preparations on each other, seeing which would indent the other. Since absolute accuracy was not necessary, the preparations were made into a limited number of groups, designated by Roman numerals according to their hardness.

Formation of Membranes.—It was aimed to produce films under conditions which would approach physically those of clinical use. For this purpose, the preparations were melted on a water bath. A sheet of plate glass was meanwhile warmed to from 38 to 40 C. by immersion in a water bath kept at this temperature. When the paraffin was melted, the plate was taken from the bath and the melted wax poured on the moist plate glass and spread with a hot spatula. The plate with the film was then immediately immersed in the 38 to 40 C. water, kept there for a few minutes, and then lifted off with a spatula. This detachment of the film furnishes a preliminary idea of its general properties.

Further experience indicates that the method of preparing these films does not affect materially their significant behavior to temperature limits. Any method that yields fairly thin films may therefore be employed. At present, I make the films by pouring a teaspoonful of the melted wax on the surface of water at about 40 C.

Determination of Strength of Film.—The films were placed in a bath at 38 C. and then gently manipulated by kneading and pulling, noting their coherence, thinness of membranes that could be formed from them; the ease with which they are torn on pulling, etc.

Determination of Breaking Temperature.—The films were immersed in a bath of a given temperature for a few minutes and then bent on themselves. At the lower temperature, this causes the film to break sharply at the crease. At the higher temperatures, the films can be doubled without breaking.

The temperature at which breaking just occurs varies for each wax, and appears to be an objective and very useful index of its fragility. Since great accuracy was not necessary, the temperatures were determined only approximately, and at intervals of 5 degrees. In the table, the lower temperature is that at which the film breaks; the upper temperature that at which it can be bent without breaking.

Determination of Ductility Temperature.—The films are placed in warm water and pulled. The water is gradually cooled, noting the temperature when the film breaks with a straight fracture, without stretching.

The lower the temperature at which the film begins to break on bending or pulling, the greater is its pliability or ductility.

The individual preparations and their properties are shown sufficiently in the accompanying table. It is probable that clinical trials will introduce new factors so that the usefulness of the various preparations cannot be judged altogether from the physical results given in this paper.

(The cost of these preparations is low; paraffin, the principal ingredient, is at present about 15 cents per pound.)

For purposes of simplification the different preparations can be arranged into a number of groups. The most distinctive feature is the hardness, ranging from the stiff beeswax to the gelatinous petrolatum. It is this property, more than any other, which determines the mechanical usefulness of the individual preparations for special purposes. Aside from this, it is desir-

able that the preparation should have a low melting point, and that it should remain pliable at a relatively low temperature.

With these points in mind, the following grouping appears most promising; it is arranged in descending order of hardness, giving the number of formulas under each class, in the order of preference:

Class I: Paraffin, Formulas, 17, 18 and 16.

Class II: Paraffin-wax mixtures, Formulas 11, 12, 10, 19, 15 and 6.

Class III: Paraffin-Asphaltum mixtures, Formulas 25 to 27.

Class IV: Paraffin-oil mixtures, Formulas 13, 23, 24, 8, 14, 9 and 4.

Class V: Paraffin-petrolatum mixtures, Formulas 5, 1, 2 and 3.

Class I. — *Simple Paraffins (17, 18, 16).* — These comprise the commercial paraffins of melting points of from 48 to 53 C. They are quite hard (generally IV of the scale), and break between 25 and 30 C. They would be used when a relatively stiff film is desired, which would separate clean from the wound. Their mechanical properties are quite similar to Ambrine. They are perhaps somewhat more fragile, but the difference does not seem important. They are the simplest and cheapest agents. Presumably any available "paraffin" could be used; but when a choice is possible, samples melting close to 50 C. would be preferred. It should be understood that commercial paraffins are complex mixtures of hydrocarbons and that the various commercial brands may differ in their physical properties, such as melting point, hardness and flexibility. It is doubtful, however, whether these differences are of practical importance in the clinical use.

Class II.—*Paraffin-Wax and Related Mixtures.*— The addition of small amounts of various waxes, etc., modifies the properties of paraffin somewhat; but the modifications are relatively slight, and I doubt whether they have any real importance. I tried mixtures with beeswax, 10 per cent. (Formula 10) and 20 per cent. (Formula 19); and with 10 per cent. of one of the

PREPARATIONS AND THEIR PROPERTIES

Formula No.	Composition			Melting Point (C.)	Degree* of Hardness at 22 C.	Fragility Temp. (Break on Bending) (C.)	A. Formation of Membrane B. Strength of Film at 38 C. (Break on Kneading and Pulling)
0 =	"Ambrine"			51	III	18-24	A. Coherent; detaches easily; fairly soft B. Very good resistance and pulls very thin; still ductile at 33°
00 =	Petrolatum (yellow)			50.5	0	.....	
000 =	Paraffin (stock)			53	...	.....	
1 =	Paraffin Petrolatum	000 00	100 20	52	II	30-35	A. Greasy, crumbly and weak, but detaches easily and sufficiently intact B. Poor resistance, though better than 2
2 =	Paraffin Petrolatum	000	100 100	49.5	I	35-35	A. Weak, greasy, noncoherent; difficult to detach; unworkable B. Very weak; crumbles
3 =	Paraffin Petrolatum	000 00	100 300				A. Ointment consistency at 43 C.; too soft to use
4 =	Paraffin; Venice turpentine		110 22	52.5	II	35-35	A. Detaches well, but soft and crumbly; slightly greasy B. Soft and crumbly
5 =	Paraffin; Petrolatum		110 11	52.5	III	35-37	A. Rather weak, although sufficiently coherent to form a film B. Soft and crumbly; very weak
6 =	Paraffin; Japan wax		110 22	52	IV	35-35	A. Hard but crumbly; not sufficiently coherent B. Very crumbly (practically like 5)
7 =	Japan wax			50	IV	37- ?	A. Does not solidify promptly when undercooled; does not detach except by scraping B. Hard, brittle and crumbly; sets very slowly
8 =	Paraffin; Olive oil		110 11	52	III	35-35	A. About like 11 B. Greasy and quite crumbly; very weak
9 =	Paraffin; Castor oil		110 5	53.5	IV	30-35	A. Solid, but not very strong, and rather crumbly B. Quite brittle and crumbly; soft

10 =	Paraffin† Yellow beeswax	110 11	52.7	IV	25-30	A. About like 11 B. Fairly strong; can be pulled very thin; somewhat more brittle than "Ambrine"
11 =	Paraffin† Spermaceti	110 11	53.5	III	25-30	A. Soft, but very coherent, and detaches well; very promising B. Good, about like "Ambrine"
12 =	Paraffin† Stearic acid	110 11	53.25	IV	25-30	A. About like 11 B. About like "Ambrine"; a little harder and probably a trifle more brittle
13 =	Paraffin† Cacao butter	110 11	53	III	35-37	A. Soft, but detaches well; very promising B. Soft and breaks
14 =	Paraffin† Cacao butter	110 22	52	III	35-37	A. Not greasy, but soft and crumbly, and not sufficiently coherent B. Soft and fragile
15† =	Paraffin† Resina	110 11	53.5	V	30-30	A. very slightly greasy; detaches and coheres; but not quite as good as 16 B. Like "Ambrine," or stronger
16 =	Paraffin†		52	IV	30-30	A. Detaches beautifully; very promising B. Rather more brittle than "Ambrine," but pulls out well; ductile at 36.5°; not at 33
17 =	Paraffin "embedding." M. P. 48 C.		48	III	25-30	A. Coherent and detaches quite well B. Practically like "Ambrine"
18 =	Paraffin, "embedding," M. P. 52 C.		51.5	IV	24-25	A. Good film, but rather fragile B. About like "Ambrine," a little harder and more fragile and not quite as plastic
19 =	Paraffin† Cera flava	110 22	53.75	IV	25-30	A. Strong and coherent film B. About like "Ambrine"; slightly more brittle
20 =	"Mulene"§		52	IV	16-18	A. Coherent, but detaches with difficulty B. About like "Ambrine"; slightly more brittle
21 =	"Parresine"§		48	II	25-25	A. Soft, but coheres well B. About like "Ambrine," but slightly more fragile

PREPARATIONS AND THEIR PROPERTIES Continued

Formula No.	Composition		Melting Point (C.)	Degree* of Hardness at 22 C.	Fragility Temp. (Break on Bending) (C.)	A. Formation of Membrane B. Strength of Film at 38 C. (Break on Kneading and Pulling)
22 =	Yellow beeswax		(62-65 U.S.P.)	II	? -16	A. Dry, strong and coherent; not easily detached B. Fair resistance
23 =	Paraffin‡ Liquid paraffin	110 5	49.5	II	33-36	B. Just ductile at 36.5°; not at 38°
24 =	Paraffin‡ Liquid paraffin Beeswax	110 5 10	52	II	33-33	B. Just ductile at 36.5°; not at 38°
25 =	Paraffin‡ Asphalt varnish	110 11	48.5	II	25-28	A. Dry, very extensile, coherent, somewhat adhesive B. Just ductile at 28°
26 =	Paraffin‡ Asphalt (Trinidad or Bermudez Asphalt Cement, 1 to 3%; or Texas Asphalt, 1%)	110 1 to 3	50.5-51	IV	27-29.5	A. Very pliable, somewhat adhesive B. Just ductile at 33.36°
27 =	Paraffin‡ Texas asphalt	110 5	51.5	IV	29.5-33	A. Pliable, but too adhesive B. Just ductile at 33.36°
28 =	"Soft paraffin"		50.5	III	26.5-28.5	A. Much more ductile than Formula 16 at body temperature B. Just ductile at 28.5°; not at 26.5°
	Cacoa butter		32.8	Not determined personally		
	Stearic acid		56	Not determined personally		
	Spermaceti		(42.50 U. S. P.) not determined personally			

* V is highest; zero lowest.

† Resin and Venice turpentine do not appear to mix well with paraffin, and must be kept stirred.

‡ The brand of paraffin used in these experiments was "Parowax," a trade name applied to paraffin marketed by The Standard Oil Co. of Indiana. It is a rather hard paraffin; softer varieties would be preferable. It should be remembered that the various oil refining companies have individual trade names of their own for their products.

§ Preparations Nos. 20 and 21 are proprietary formulas.

following spermaceti (Formula 11); stearic acid (Formula 12) and resin (Formula 15). Nos. 15 and 19 have rather high melting points. The others stand so close to the simple paraffin that I doubt the advisability of giving them an extended trial. If experimentation should appear desirable, I would advise Formula 11 (the spermaceti mixture); or if this is too expensive, Formula 12 (stearic acid mixture).

The *proprietary mixtures of secret composition* also belong in this general class:

"*Ambrine* (Formula 0), as I have said, approaches very closely to the simple paraffins.[2] It is rather more plastic and less brittle; but the difference does not impress one as important.

"*Mulene*" (Formula 20) also comes very close to the simple paraffins, and to Ambrine. The same remarks apply to both.

"*Parresine*" (Formula 21) is a rather different article. It is softer and more fragile than "Ambrine," but on the whole it does not depart seriously from the paraffin type.

Class III. — *Paraffin-Asphaltum Mixtures.*—These are distinctly more pliable and more adhesive than the plain paraffin, and can be made into thinner films. Theoretically, these properties would be advantageous; practically, I doubt whether the advantages are important. The paraffin and asphalt do not form perfect mixtures, and must be kept stirred.

A mixture made with 10 per cent. of "asphalt varnish" possessed the desirable qualities, but since the composition of the asphalt furnished is complex and probably variable, no further experiments were made. Other preparations were made with the semi-solid asphalts, such as Trinidad or Bermudez "asphalt cement," from 1 to 3 per cent.; or Texas asphalt (Formula 26). These are not quite so plastic as the varnish formula, but nevertheless are quite promising. Higher proportions are less desirable, such as 5 per cent. of Texas asphalt in Formula 27.

2. In a preliminary study of paraffin mixtures in the A. M. A. chemical laboratory, Ambrine was found to contain about 96 per cent. unsaponifiable matter (paraffin).—Ed.

Class IV. *Paraffin-Oil Mixtures.*—These are considerably softer than the paraffins, and also considerably weaker (more friable); however, they are fairly coherent. They would perhaps be preferable in the early stages of treatment, since they would be somewhat emollient. The most promising is the mixture with 10 per cent. of oil of theobroma (cacao butter, Formula 13); then come the one with 5 per cent. of liquid petrolatum (Formula 23), and this with beeswax (Formula 24), and that with 10 per cent. of olive oil (Formula 8). That with 20 per cent. of cacao butter (Formula 14) is scarcely sufficiently coherent. That with 5 per cent. of castor oil (Formula 9) was unpromising. The mixture with 20 per cent. of Venice turpentime (Formula 4) had the properties of Class V, but appeared undesirable.

Class V. — *Paraffin-Petrolatum Mixtures.* — These differ materially from the other classes. They are very soft and might be termed "solid ointments." They are rather greasy, and crumble easily; No. 3 (75 per cent. petrolatum) was really a cerate and would not form a workable film. No 2 (50 per cent. petrolatum) would also be practically unworkable. Twenty per cent. petrolatum (Formula 1) and 10 per cent. petrolatum (Formula 5) form weak, but manageable films. The last (Formula 5) would be worth trying when a very soft film is desired, for instance, on very sensitive surfaces.

Application to the Skin.—A selected series of preparations were applied to the skin in the same manner as they would be used clinically. A strip of skin about an inch wide was painted with the melted wax; on this was laid a very thin layer of cotton and over this was painted another layer of the wax. The adjacent strip of the skin is now painted with a second preparation, and so on. (This will be a very suitable method of comparing the preparations clinically.) The strips are covered with a bandage and left on for at least an hour. The following presented no marked differences:

"Parawax" Paraffin.....(16)
Paraffin-spermaceti .....(11)
Paraffin-stearic acid..... .....(12)
Paraffin, 48°.....(17)
Paraffin-theobroma .....(13)
"Ambrine" .....(0)
Paraffin-petrolatum .....(5)
"Mulene" .....(20)
"Parresine" .....(21)
Paraffin-beeswax .....(10)
Paraffin-resin .....(15)
Paraffin-liquid Paraffin.....(23)
Paraffin-beeswax-liquid paraffin...(24)
Paraffin-asphalt mixtures..(25 and 26)

Paraffin-asphaltum gave a film that was somewhat adherent, but that was smooth, strong and so pliable that it could be wound about a pencil without cracking.

CONCLUSIONS

The preparation and mechanical properties of a series of paraffin-film mixtures suggest that the most important mechanical property of such films from the therapeutic standpoint is their hardness. It is suggested that several degrees of hardness might possess advantages under different conditions.

Surgeons who desire to experiment with the paraffin treatment of burns are urged to use simple preparations of known composition, so that their results can be compared, and so that any deficiencies may be met, and improvements made intelligently.

The physical and mechanical properties of a series of paraffin and mixtures are described. *Ordinary paraffin*, melting between 48 and 53 C. (118-128 F.), preferably about 50 C. (122 F.) appears to possess practically the mechanical properties of the French preparation, and is urged as the standard of comparison.

Paraffin-Asphaltum (Formula 26) gives a preparation of superior pliability.

The following additional formulas are suggested for clinical trial as preparations of increasing softness:

Paraffin-Spermaceti (Formula 11): Paraffin, 10 parts; spermaceti, 1 part.

Paraffin-Theobroma (Formula 13): Paraffin, 10 parts; theobroma oil, 1 part.

Paraffin-Petrolatum (Formula 5): Paraffin, 10 parts; yellow petrolatum, 1 part.

In comparing these films with each other, or with proprietary formulas, claimed points of superiority should be clearly established. Finally, experience may show it to be advantageous to add to the simple combinations I have suggested one or more medicinal agents such as resorcin, eucalyptus, scarlet red, etc.

THE PROVOCATION OF THE LUETIN TEST IN NONSYPHILITIC PATIENTS *

H. N. COLE, M.D.

AND

H. V. PARYZEK, M.D.

CLEVELAND

(*From The Journal A. M. A., April 14, 1917, p. 1089*)

An exact cutaneous test for syphilis is greatly desired by syphilologists. From a practical diagnostic standpoint, an easy, exact and cheap cutaneous test for syphilis is a thing much to be hoped for.

As early as 1909, Charlet, Gautier, Favre and Nicholas had experimented with congenital syphilitic liver extract, which they termed "syphilin," and like experiments had been tried in Neisser's and Jadassohn's clinics and in several others. Fischer and Klausner, with the same idea, had also worked with "pallidin," a suspension made from pneumonia alba (the lungs of congenital syphilities rich in *Spirochaeta pallida*).

Noguchi's[1] report on his luetin reaction was received, therefore, with satisfaction, and the test is used quite extensively in this country and more or less abroad.

In attempting to explain its mechanism, Neisser noted that the skin of patients with late syphilis reacted not only to the luetin test, but also with many other foreign bodies. On their being injected intradermally, there was a reaction much like the luetin test which Neisser termed an *Umstimmung* of their skin.

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* This investigation was undertaken at the suggestion of the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Noguchi, Hideyo: Jour. Exper. Med., 1911, **14**, 557.

Boas and Ditlevsen[2] have reported that they were able to produce the luetin reaction with gonococcal and colon bacillus suspensions in late syphilis without a gonorrheal infection or gastro-intestinal symptoms being present. They also found that with the "luetin control," which contains the culture mediums of agar-agar and ascitic fluid but no *Spirochaeta pallida*, in a series of tertiary and hereditary syphilities, they were able to obtain nearly as many positive reactions as had been found with the luetin itself, and of almost the same intensity.

This *Umstimmung* of the skin of patients with old syphilis is not enough in itself to discredit the luetin reaction as a practical therapeutic test; but it might be found that these patients would react to some cheap substance in a way which would be of great help in diagnosis and guidance in treatment, especially if normal persons did not so react. This, however, is the especial criticism that we wish to raise. In the first place, Boas and Stürüp,[3] in 1914, working with an extract of chancroidal bubo, found that they were able to achieve Klausner's results with pallidin. Secondly, a number of workers, including ourselves, have noted an occasional positive reaction in a normal person. Two delayed pustular reactions produced by Noguchi's original luetin in medical students are shown in the accompanying illustrations. These men had repeated negative Wassermann reactions with absolutely negative histories and physical findings. Thirdly, Sherrick[4] has reported that an involuting luetin reaction can be revived by the use internally of potassium iodid, or that intradermal injections of a 0.5 per cent. suspension of agar gives rise to a reaction indistinguishable from the luetin test after administration of potassium iodid. Moreover, a nonsyphilitic person taking potassium iodid or having taken it some time before, up to a space of three weeks in his experience, generally responds with a positive test to the

2. Boas and Ditlevsen: Arch. f. Dermat. u. Syph., 1913, **116**, 852.
3. Boas and Stürüp: Arch. f. Dermat. u. Syph., 1914, **120**, 730.
4. Sherrick, J. W.: The Effect of Potassium Iodid on the Luetin Reaction, The Journal A. M. A., July 31, 1915, p. 404.

luetin reaction. This work has been substantiated by Kolmer, Matsunami and Broadwell[5] and others. In addition, Stokes[6] has found that many normal persons react to intradermal injections of emulsions of normal and pathologic skin, agar-agar and bismuth subnitrate. The purpose of our investigation was to determine especially whether the provocation of the luetin reaction in nonsyphilitic persons is specific for potassium iodid, or whether other substances may have a similar action. This is a matter of some interest from a diagnostic standpoint, for evidently the presence of all provocative substances should be excluded when a diagnostic luetin test is to be made.

Dr. Sollmann also suggested that the knowledge of the iodid-luetin and related reactions may contribute to the understanding of the mechanism of the iodid action in syphilis and iodism.

Incidentally, we also gathered information as to the frequency of the occurrence of the luetin reaction in normal persons controlled by the Wassermann test, and on the influence of the dosage of the iodid.

Our first work was done with potassium iodid, the usual technic being to make a luetin[7] and Wassermann test, and if negative, to give the patients the drug in 10 or 15 grain doses and upward, three times a day after meals from three to five days or a week, and then apply the luetin test. The accompanying table shows the doses used and the duration of their administrations.

Out of eighteen such cases, fourteen reacted with strongly positive, papular and pustular reactions; two of them were negative, and the other two gave strongly positive reactions to the control test. One of these was a case of a cerebellar cyst with a negative lumbar puncture which afterward came to operation and

5. Kolmer, J. A.; Matsunami, Toitsu, and Broadwell, Stuart: The Effect of Potassium Iodid on the Luetin Reaction, The Journal A. M. A., Sept. 2, 1916, p. 718.

6. Stokes, J. H.: Jour. Infect. Dis., 1916, **18**, 402, 415. We are indebted to Stokes' careful articles for a portion of the historical material on the luetin reaction already quoted.

7. The luetin used in this investigation was kindly furnished by the H. K. Mulford Company.

showed absolutely no signs of syphilis. The other case was one of typhoid with no history or evidence of syphilis and with a negative Wassermann test.

We have, therefore, substantiated the work of Sherrick, Kolmer and others, that (1) some non-syphilitics respond to luetin spontaneously, and (2) in

Fig. 1.—Delayed pustular reaction in normal medical student; control not visible.

those nonsyphilitics who do not respond spontaneously, the reaction can generally be provoked by iodids.

Our next problem was to determine whether or not other drugs could provoke the reaction and positive results be attained with bromids and nitrates, which are closely related to the iodids.

Of eight patients on potassium nitrate, five gave strongly positive reactions, one faintly positive, and two negative. With potassium bromid, there was one strongly positive reaction and seven weakly positive out of eight patients tested. We did not try the sulphocyanid ion because of its toxic qualities.

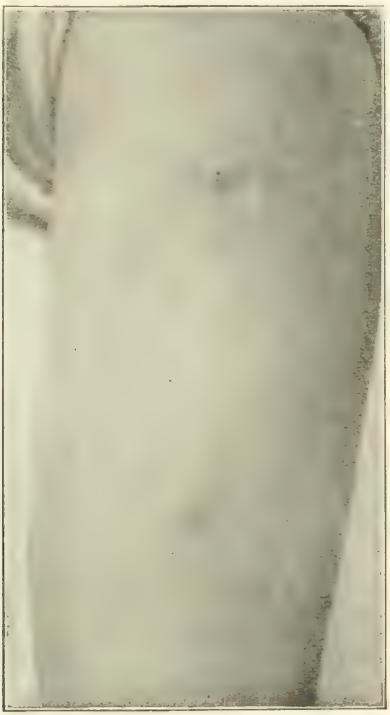

Fig. 2.—Delayed pustular reactions in normal medical student; control not visible.

We thought we would try the drug, using another positive ion instead of potassium in the form of the iodids and bromids. The one patient put on sodium iodid gave a strongly positive reaction; the same was also true of one patient on calcium bromid. Out of three patients put on sodium bromid, one had a strongly positive reaction and two weakly positive.

SUMMARY

Out of thirty-nine cases tested by the luetin reaction, two normal persons gave pustular reactions to the control test. Among eighteen taking potassium iodid, sixteen gave positive reactions, these being most strongly positive in those patients who had received from 200 to 600 grains.

This action is found not to be specific for potassium iodid alone, as it was caused, though in a lesser degree, by sodium bromid in three cases tested, by potassium nitrate in six out of eight cases tested, and by calcium bromid and by sodium iodid each in one case tested.

In a future communication we expect to publish the results of our experiments with organic iodin compounds. Perhaps then we shall be in a better position to draw some conclusions as to the action of iodin and as to the syndrome known as iodism.

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DEVELOPMENTS IN THE PARAFFIN TREATMENT OF BURNS AND OTHER OPEN WOUNDS*

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(*From The Journal A. M. A., June 16, 1917, p. 1799*)

Further experience in the laboratory and reports of clinical applications are showing pretty clearly that some of the current endeavors in the paraffin treatment of burns are proceeding in directions that are not likely to lead to improvements; they have also indicated the directions in which further experimentation is desirable, and have already led to certain improvements that appear of sufficient practical importance. All of these considerations may justify the publication of this note.

PHYSICAL CRITERIA

The important qualities of paraffin for burns, as mentioned in my previous paper,[1] and in that of Leech[2] are the properties of pliability, ductility, low degree melting point, hardness and adhesion. I should be inclined to rate the importance of these properties in the order given; but all the properties must be within certain limits.

I have devised methods for testing these properties. These were described in the paper of Leech. They do not entirely eliminate the personal factor; but they are sufficiently quantitative for practical purposes, and are so simple that they can be easily applied. The factor of adhesion has not yet been measured quantitatively, but is probably of slight practical importance.

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* From the Pharmacological Laboratory of the Western Reserve University, School of Medicine.

1. Sollmann, Torald: Suggested Formulas for Paraffin Films, THE JOURNAL A. M. A., April 7, 1917, p. 1037.

2. Leech, P. N.: "Ambrine" and Paraffin Films, THE JOURNAL A. M. A., May 19, 1917, p. 1497.

The Addition of Other Waxes, Etc., to Paraffin.—In my previous paper, I expressed the opinion that simple paraffin would accomplish everything that could be accomplished by any of the mixtures (those made by myself and various others that are on the market, or have been submitted to me by their inventors); and that the most promising direction for improvement would be in the choice of the paraffin itself, rather than in additions. Further experience has established the correctness of these predictions. There has now been considerable experience with simple paraffin, and with various mixtures.[3] This has shown that they are clinically indistinguishable. A review of my own data on the effect of other waxes added to paraffin shows that the essential properties were not improved by such additions, except that asphalt does make the waxes a little more adherent.[4]

The addition of rosin, which is advised in so many of the published formulas (evidently because of the misleading statements concerning the composition of "Ambrine"), is distinctly detrimental. The rosin appears insoluble in paraffin, and therefore never makes a good mixture. The addition of oils lowers the melting point, but practically destroys the ductility and pliability.

The effects of the various additions are deduced from my first pages as follows:

Asphalts sometimes improve pliability and ductility and lower melting point.

Naval pitch improves pliability, not ductility, and raises the melting point.

Rosin raises the melting point and diminishes ductility, but has no effect on pliability.

Beeswax, spermaceti, stearic acid, linseed oil, Venice turpentine, olive oil, castor oil, theobroma and Japan wax diminish ductility and pliability.

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3. The clinical applications were made by Dr. J. R. Beiter of Canton, Ohio, who expects to report his observations in more detail.

4. Dr. Leech and I have both noticed that the addition of even traces of asphalt to paraffin greatly lowers its surface tension, so that when the melted mixture is poured on warm water it forms a thin, even film, whereas simple paraffin tends to collect into rather thick drops.

Petrolatum diminishes pliability and ductility, but makes no change in the melting point.

Liquid petrolatum[5] lowers the melting point and diminishes ductility and pliability.

PHYSICAL IMPROVEMENTS IN PARAFFIN

The paper of Dr. Leech shows that several refineries produce paraffins that surpass greatly for these properties the ordinary pharmacopeial paraffin. A glance at his paper shows those brands that are most useful. I am of the opinion that paraffins intended for the treatment of burns, etc., should comply with certain specifications as follows:

Paraffin for Films.—Paraffin for use on burns, etc., should be solid, but more ductile and pliable than the official paraffin, and have a rather lower melting point. When intended especially for this purpose, it should be liquid at or below 50 C.; a thin film, when prepared and tested by the methods devised by me and described in the paper of Dr. Leech, should be pliable at 28 C., and ductile at 31 C.

I would recommend that in future only paraffins complying with these specifications be used in the treatment of burns. A number of these are enumerated in Leech's paper, and apparently there is no difficulty in refineries producing other brands should they be desired. I believe that these should have the preference over the more complicated and rather inferior mixtures, whether these are proprietary preparations or published formulas, unless a definite advantage can be shown for the latter. As I have previously said, all the experience I have gathered goes against any such advantages, and I consider experimentation along that line as entirely unpromising. For cosmetic purposes, it may be advantageous to give a flesh tint to the paraffin, by the addition of a trace of scarlet red or sudan.

5. The brand of liquid petrolatum used throughout these experiments was "Stanolind Liquid Paraffin."

ADDITION OF ANTISEPTICS, ETC.

A number of the published formulas contain added antiseptics, such as resorcin; betanaphthol; epithelial stimulants like sudan red; and aromatic oils like eucalyptol. The last is added confessedly as a mere perfume, and doubtless performs that purpose to some degree. The others — the antiseptics and stimulants — must be absolutely useless. Solid paraffin is used familiarly as a hermetic seal for chemicals. It has been found perfectly successful for that purpose, and it does not lose its efficiency when the substance is incorporated with it. In other words, the film of solid paraffin encloses the added antiseptic, etc., tightly and nearly as hermetically as if it were inclosed in a rubber bag. Only an infinitesimally thin layer at the very surface can diffuse into the wound.

I have shown this by adding resorcin or phenol to melted paraffin, and pouring this in films of about 2 to 3 mm. thickness. The films were then immersed in water.

With resorcin, 0.2 per cent., in paraffin;[6] or 0.2 per cent. in paraffin, 2 parts, liquid petrolatum, 1 part, after five days the water contained no trace of resorcin.

With 1 per cent. phenol (carbolic acid) in paraffin: In two and a half hours there was a very faint trace in the water, which did not increase in five days. The wax when melted with water showed an abundance of phenol.

LIQUID-SOLID PARAFFIN SEQUENCE

Perhaps the greatest difficulty in the use of the paraffin treatment is in the application of the first coat of the melted paraffin. This is apt to be painful if the paraffin is at all overheated — and this is often difficult to avoid in practice. The use of a brush with melted paraffin is apt to be somewhat painful, and spraying apparatus for melted paraffin is not very satisfactory.

We have found that these disadvantages can be avoided by applying for the first coat liquid petrolatum in place of the melted paraffin.

6. In these experiments, the "Parowax" brand of paraffin was used.

Otherwise, the application is quite as usual: The cotton film is laid on the liquid petrolatum; then the melted paraffin is painted over the cotton, etc.[7]

Clinically, this method is highly satisfactory. The liquid petrolatum is entirely painless, and protects the wound against excessive heat from the melted paraffin. The liquid petrolatum can be easily applied with an ordinary oil atomizer. However, this is not at all necessary, a thin layer being easily applied with a cotton swab. Dr. Beiter expresses himself as especially enthusiastic over this modification.

Use of Antiseptics, etc., with Liquid Petrolatum.—The use of the liquid petrolatum for the first coat has the further advantage that it promises the effective application of anesthetics, antiseptics or stimulants to the wound. These would simply be dissolved in the liquid petrolatum (or if insoluble in this, in olive oil). I have not yet given this method a clinical trial, but have worked out a series of solutions which are very promising. These will be enumerated in the next section, so that they may be tried more extensively, and in this way any improvements in the treatment of burns may be hastened. It is scarcely necessary to call attention to the fact that under the present circumstances prompt improvements in these directions would be especially desirable.

COMPARISON WITH OTHER METHODS OF TREATMENT

Almost all surgeons who have had occasion to employ the paraffin treatment express themselves with various degrees of enthusiasm as to its advantages over the older methods of treatment. However, no exact comparisons have been published, so that it is difficult to say how much, if any, of the present enthusiasm is due to novelty. It would be desirable that actual comparisons be made between these methods, by those who have the opportunity and sufficient interest. Such experiments are already being started

7. It would probably be a considerable convenience if manufacturers of absorbent cotton would market for this purpose rolls consisting of single layers, of the proper thickness, and about 6 inches wide.

by several workers; but their value is likely to be proportioned to the number of workers engaged; again, especially because early results are particularly valuable.

I subjoin a list of comparisons that seem to me especially desirable:

I would suggest either plain paraffin, or the liquid-solid paraffin sequence as a standard of comparison. This would be applied, together with the methods to be compared, to adjacent or contralateral areas. If applied to adjacent areas, the wound should be divided by imaginary lines into zones about 2 inches wide (oriented by marking the skin outside of the wound). It is important that the methods applied to the same patient should not be so numerous as to interfere with the practicality of the application. If it is at all possible, photographs should be taken.

LINE 1.—*Local Anesthetics.*—This appears one of the most promising improvements. I would suggest that the following be tried out, generally dissolved in liquid petrolatum, and applied by the liquid-solid sequence:

Chloretone, from 1: 200 to 1: 1,000 (soluble in about 200 parts of liquid petrolatum).
Orthoform-New, a 1 per cent. suspension made by dissolving the orthoform in a little alcohol and shaking with olive oil. The orthoform is but little soluble in either liquid petrolatum or olive oil.
Camphor, from 1: 100 to 1: 20 (it is completely soluble in 20 parts of liquid petrolatum; incompletely in 10 parts).
Menthol, 1: 10 (completely soluble).

LINE 2.—*Antiseptics and Epithelial Agents.*—These may have a field, especially in the later stages, or with slowly healing ulcers. I would suggest the following:

Eucalyptus oil, 2 per cent. (completely soluble).
Resorcin, 1 per cent. in olive oil (nearly insoluble in liquid petrolatum).
Betanaphthol, 1: 400, dissolved in a little alcohol and shaken with liquid petrolatum (it is nearly insoluble in liquid petrolatum).
Gentian violet, 1: 5,000 suspension (dissolved in a little alcohol and shaken with the liquid petrolatum; it is nearly insoluble in liquid petrolatum).
Scarlet red, 0.5 per cent. suspension in liquid petrolatum (only slightly soluble).

LINE 3.—*Comparison of Paraffin with Astringents and Washes.*—This and the following represent comparisons of the new with the older methods, to arrive at a definite judgment of their relative advantages:

Picric acid, saturated solution.
Solution of aluminum acetate, N. F., diluted ten times.
Boric acid, 2 per cent.
Sodium bicarbonate, 2 per cent.
Lime water.
Magnesium sulphate, 5 per cent.
Potassium sulphate, 1 per cent.
Tincture of myrrh, diluted with 8 parts of water.

LINE 4.—*Comparison of Wax Treatment with Other Fatty Methods.*—

Petrolatum, applied on gauze.
Fisher-gauze and cotton.
Liquid petrolatum gauze and cotton.
Carron oil on cotton: Cotton film, paraffin and cotton.
As the preceding, sesame oil being used in place of linseed oil, in making the lime liniment.

LINE 5.—*Comparison of the Waxes.*—While present experience does not incline me to attach much importance to modifications of the paraffin by the addition of other waxes, any one interested in this phase of the subject may find the following most promising:

Paraffin.[8]
Paraffin with Texas asphalt, 1 per cent.
Paraffin with spermaceti, 10 per cent., or paraffin-stearic acid, ten per cent.
Paraffin with theobroma, 10 per cent.
Paraffin with liquid petrolatum, 10 per cent. (paraffin 90, liquid petrolatum[5] 10).
Paraffin with liquid petrolatum, 33 per cent. (paraffin 67, liquid petrolatum[5] 33).
Formula published by Leech:

Paraffin (M. P. by U. S. P. method 47.2 C.)	97.5 gm.
Asphalt varnish	from 3 to 5 drops
Olive oil	1.5 c.c.

CONCLUSIONS

Several refineries prepare paraffins of low melting point that are superior in their physical properties to any of the mixtures on the market.

The addition to paraffin of waxes, resins, etc., as also of antiseptics, etc., is superfluous.

Further improvements are not to be sought along these lines.

8. For these experiments "Parowax" could be used. This brand of paraffin, however, melts a little higher than 50 C. It would seem, therefore, that the more suitable grades of paraffin, such as are described in the recent Report of the A. M. A. Chemical Laboratory should be employed in the preparation of these mixtures.

The difficulties of the paraffin method are greatly reduced by employing liquid petrolatum instead of melted paraffin for the first coat. Further improvements may be attempted by the addition of antiseptics, stimulants, and especially anesthetics to the liquid petrolatum of the first coat.

Systematic comparison of these improved methods with the older methods is desirable.

PARAFFIN IN THE TREATMENT OF WOUNDS AND BURNS

OBSERVATIONS ON VARIOUS PREPARATIONS

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(*From The Journal A. M. A., June 16, 1917, p. 1801*)

The successes claimed for "Ambrine" in the treatment of burns promised a large field in industrial accidents and therefore prompted extensive trials. Owing to my inability to obtain "Ambrine," various paraffin compounds were used — formulas containing eucalyptus, resorcin, betanaphthol, resin, cera flava, olive oil, and scarlet red, in ordinary paraffin. My series of cases represents over 4,000 wax dressings on every conceivable burn, and many lacerated wounds.

Prior to the employment of the wax treatment of burns we had employed the usual methods — various ointments, various aqueous solutions, the bath treatment, exposure to the air and picric acid.

Our technic in the use of waxes was as follows: All burns were carefully cleaned at our emergency hospital by well trained men, any blebs were opened, and all the skin that could be taken off with ease was removed. The burned area was dried either by exposure to air or by gently wiping the surface with cotton pledgets dipped in ether. Over the involved area a thin film of the wax was painted. (The wax is kept constantly in a water bath, so that it is at all times ready for instant use.) Over the wax film a thin layer of cotton or a split piece of sheet wadding was placed and a second film of wax was painted, sealing it to the skin at the edges of the cotton dressing. Over this a heavier cotton dressing was applied and

then the bandage. We found that if the injured surface was wet or damp the first paraffin film would not adhere.

We began with the various paraffin mixtures enumerated above; but we failed to see any differences, except some disagreeable features with the resin mixtures. For example, the undissolved resin sank to the bottom of our warming receptacle and injured the brush with which the wax was applied, making the application to the injured surface painful. We therefore discarded all drugs in our wax and used the commercial "Parowax," applied as above. This was sometimes tinted pink with scarlet red simply for cosmetic reasons.

The question of melting point was at first an important one, because to apply a hot solution to a large area of denuded nerve endings usually brought a prompt and energetic reaction, and to wait until the wax cooled to the extent that a film formed over it meant that it would cool below the liquid state before it could be applied. However, the water bath or household double boiler holds the melting point very well.

A suggestion made to me by Dr. Torald Sollmann has eliminated the importance of melting point temperature entirely, and greatly simplified the dressing as well as adding to the comfort of the patient at the time of the dressing, and in no way changing the results. His suggestion was that the wound be painted with liquid petrolatum, further treatment proceeding as with the wax. In this method a layer of liquid petrolatum and then the cotton or sheet wadding are placed over the injured area before the warm paraffin is painted on the dressing. This method has been followed in all of our recent cases and is greatly appreciated by the patients, who are the court of last appeal. It is essential that the dressing adhere at least to the skin about the edges of the dressing; otherwise the secretions are pouring out over the intact skin and excoriating it, soiling the dressings and making a disagreeable odor.

ADVANTAGES OF THE WAX TREATMENT OF BURNS

1. It is an inexpensive dressing; a pound of wax and a pint of liquid petrolatum, together costing about 65 cents, will dress many burns. It replaces the gauze which at this time is quite expensive.

2. It is a comfortable dressing, because it is firm and smooth, and the granulating surface does not grow through it as with the gauze. The paraffin is hard enough to make the dressing somewhat rigid and acts as a splint.

3. It is a cleaner dressing than any I have used because the wound discharge is not permitted to soak through the impermeable wax covering, soiling all the linen that comes in contact with the patient. As the secretions are sealed up, there is no noticeable odor about the patient, which was a disagreeable factor with former methods of treating these injuries.

4. Superficial burns heal more readily under the wax treatment than with any other method with which I am familiar. This is due to but one fact: Under former methods of application of solutions and oily substances, no matter what their kind, the granulations penetrated the meshes of the dressing in contact with the wound, and on removal at redressings, these granulations were destroyed, regardless of the care with which the dressing was done or the method employed in the removal of the dressings in contact with the wound. The paraffin film method does not adhere to the injured area, and therefore does not injure the granulation tissue and the epithelium that is attempting to cover in the denuded area. Early in the course of the burn, if it is an extensive one, the entire sealed surface of the dressing will be filled with fluid so that it is merely lifted off. Later, as the wound heals, the secretion diminishes, and the granulations begin to grow, the epithelial islands appear as white points at the site of hair follicles, and from these islands the epithelization takes place rapidly because the epithelium is not injured in the dressings and redressings.

5. Deep burns do not repair any more rapidly under this method than any other method. There is no difference in the scars of burns treated by the wax method and any other method. If the true skin is destroyed, the end-result is scar tissue or an ulcer. If scar tissue replaces the destroyed tissue, it performs as does scar tissue that develops under any and all forms of treatment, and as scar tissue has performed since the beginning of time. We have tried treating two sides of a body burned to about the same degree, with the wax method, the solution method, and various other methods, and have been unable to detect any difference in the end-results, as to scar.

6. The wax method is much more comfortable at dressing time than any other method with which I am familiar, for the purely mechanical reason that the granulations do not grow through it, and it is lifted off painlessly. To those who have to do with burned men this means a great deal. The pain endured by the patient as the dressings were removed under previous methods of treating burns left an unpleasant impression to carry with one on the day's rounds.

7. We think there are fewer furuncles on our burned patients since the wax has been used, but nephritis is quite as common.

DISADVANTAGES

1. Some patients refused to be treated with the wax when we were applying the warm wax directly to the injured area, because of the pain. These complaints are no longer heard since the liquid petrolatum has been used for the first coat.

2. Owing to the fact that this method has received so much favorable comment in the lay press, as to negligible scars, perfect comfort on application and other extravagant statements, the man who uses it for the first time will probably be disappointed.

3. This method is a time consumer; it requires more care and patience than do the dressings by other methods.

CONVENIENT DEVICES FOR MELTING PARAFFIN FOR BURNS*

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CLEVELAND

(*From The Journal A. M. A., June 23, 1917, p. 1895*)

One of the main drawbacks in the paraffin method is the inconvenience of melting the paraffin and keeping it melted at the proper temperature, between 55 and 60 C. This is almost impossible by direct heat, since this would tend to overheat, and would also cool too rapidly. The device ordinarily used is a double boiler (cereal boiler). This answers, but is far from ideal. For simple dressings, as in office practice, it is inconvenient. With extensive dressings, as in shop practice, there is the further difficulty that the paraffin will either overheat or cool unduly while the attention of the surgeon is centered on the patient. The temperature could, of course, be kept constant by the ordinary scientific thermostats, but these are not practical, since they are too cumbersome, are immovable and are easily injured. Under the stress of war practice, the difficulties must be still greater.

I have experimented with two devices which are simple and appear practical: an electrical "food warmer" for office use; and an "acetate thermostat" for shop and field practice.

1. Food Warmer: This is the pint size sold in the stores for the warming of babies' milk bottles. When filled one half or two thirds with paraffin of melting point, 47.5 C., it can be used in three minutes after

* From the Pharmacological Laboratory of Western Reserve University, School of Medicine.

* Research partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

the current is turned on. If the current is then turned off for two minutes, it will have just the right temperature. It will remain usably melted for ten minutes without current, when a crust begins to form; it can then be again made usable by turning on the current for a few moments.

This should be particularly useful when the paraffin is used only occasionally, as in ordinary office practice.

Table 1 gives the data of an actual experiment.

2. Acetate Thermostat: Sodium acetate has the curious property of melting and congealing at just about the temperature that is needed for the application of the paraffin.[1] It retains this temperature constant for a long time while passing from the liquid to the solid state. For instance, some sodium acetate melted in a crucible remained at the temperature of 58 C. for an hour and ten minutes without the application of further heat.

This is shown by the following data:

TABLE 1. PARAFFIN IN FOOD WARMER

Current	Temperature of the Paraffin (Degrees C.)	Remarks
On for 3 min.....	65.5	Nearly completely melted, except a crust around the margin; center usable, but rather hot
Current turned off		
3 min.	58.5	
9 min.	50	Still usable
20 min.	48	Pellicle of solid paraffin
36 min.	47.5	Not usable; mostly solid
Current turned on		
2 min.	66.5	Mostly melted; usable, but hot
Current turned off		
13 min.	48	Pellicle; just usable

Two nickel crucibles of the same size were filled, the one with 40 c.c. of water, the other with sodium acetate. They were heated to 68.5 C. The heat

1. This property was brought to my attention by a report of the Chemical Laboratory of the American Medical Association (Annual Reports of the Chemical Laboratory of the American Medical Association. **9**, 105) on sodium acetate in warming bottles.

was then withdrawn, and the temperature noted from time to time as cooling proceeded (Table 2).

When sodium acetate is used to fill a pot surrounding a vessel containing paraffin, it keeps the paraffin melted at just the right temperature for application for three hours after the pot has been removed from the fire. If in the meantime it has been set into a fireless cooker, the time could of course have been further prolonged.

TABLE 2.—COOLING OF SODIUM ACETATE

Time (Minutes) After Removal of the Heat	Temperature of Sodium Acetate (Degrees C.)	Temperature of Water (Degrees C.)
1	66.8	66.5
6	61	54.5
10½	56.5	48.5
13	56.5	45
15	57.8	
17	58	41.5
21	58	37.5
26	58	31.5
60	58	26.5
75	57	25
90	51 (solid)	24

TABLE 3.—PARAFFIN IN ACETATE THERMOSTAT

The pot was removed from the fire at 10:20 a. m. and set on the table.

Time After Removal from Fire	Temperature of the Melted Paraffin (Degrees C.)	Remarks
6 minutes	59	Ideal for use
16 minutes	58	
28 minutes	55	
45 minutes	54.5	
2 hours	53.5	
2 hours, 45 minutes	50	Faint film; usable
3 hours	48.5	Continuous film; just usable
3 hours, 15 minutes	48	Partly liquid; but not usable
4 hours	47.5	Mostly solid; not usable

The advantages of this will be appreciated at once: the paraffin needs no further attention after it has been melted, until three hours afterward, and then it can be quickly reheated. The paraffin pot can be carried to any part of the shop, or in an ambulance to the dressing station. The sodium acetate is syrupy, and does not easily spill; it does not evaporate, so that no attention is required in this direction; indeed, the outer and inner vessels could probably be joined by solder.

The device that I am using, which appears especially practical, consists of an ordinary glue pot of size O. The outer pot is filled two thirds with official sodium acetate (requiring probably something over a pound). The inner vessel holds about a pound of paraffin.

In Table 3 are the data of an experiment, showing the rate of cooling.

ON THE USE OF TRYPSIN PREPARATIONS

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(From the Journal of the American Pharmaceutical Association, September, 1917)

Trypsin preparations have found some use in medicine for many years, and mostly in the way of internal administration. This use is greatly limited by the low digestive value of the products that have been available up to the present time, which, with a few exceptions, have been weak.

In the manufacture of digestive ferments the production of trypsin has not kept pace with that of pepsin, the practical isolation of which on the commercial scale has reached a remarkable degree of thoroughness. Indeed, it seems now to be the custom among the leading manufacturers of pepsin to make first a product of far greater strength than that required by the Pharmacopoeia and dilute it down to a constant value, as needed, by the addition of some inert substance.

Nothing of the sort has happened with trypsin. A very limited number of manufacturers, here and abroad, have attempted to put on the market a reasonably strong product under the name of trypsin, but many so-called pancreatins have been made, the activity of which is amylolytic rather than proteolytic. The assay method of the Pharmacopoeia is directed practically to the determination of the starch-converting power of the pancreatin, while the milk peptonizing test is merely a limit test, and a very unsatisfactory one at that. A stimulating service might be rendered if the Pharmacopoeia were to call for a more stringent proteolytic test than the alteration of the casein in 400 c.c. of milk by 0.28 gm. of pancreatin in half an hour. The test is based on the assumption that market milk contains 3.5 per cent. of casein, which, however,

is not the case, as milk is now produced for fat value rather than for total solids or casein. With the average market milk compliance with the test would simply indicate that one part of pancreatin is able to convert about forty times its weight of casein to the stage where it is not coagulated by acetic acid, and this is a low requirement because only superficial alteration of the casein is required to exhibit this change in behavior.

The practical value of the great mass of these pancreatins is questionable, because of their low degree of activity. Of the actual concentration of the pancreas enzymes in the intestine we know but little, and it is possible that there are times when the ingestion of the ferment might be of great service. Of the limited use for pepsin there can be no doubt, since abundant observations of stomach contents in recent years have shown that the ferment is practically always present. The lack of acid is far more frequent. But failure in the proper functioning of the pancreas, as far as the secretion of ferments is concerned, is not rare and hence the therapeutic use of commercial ferments.

This brings up the question of the administration, which from several points of view, is an important one. To be of use as aids in intestinal digestion pancreas ferments given by the mouth must pass through the stomach. It has been long held that trypsin and amylopsin suffer deterioration or even destruction in contact with the gastric secretion through the action of acid and pepsin. This view seems to be based largely on statements of Kuehne and other earlier workers, and it is only in recent years that the question has been more closely studied. In our laboratory much attention has been given to the problem, and the following facts have been brought out:

a. It is necessary to distinguish between the action of acid alone on trypsin and acid plus pepsin.

b. When digested at body temperature with low concentrations of hydrochloric acid trypsin is practically not much weakened, even when the duration of

the incubation is extended through an hour, with the concentration of the "free" acid from 0.2 to 0.3 per cent.

c. The case is very different, however, with pepsin present. Acid and pepsin working together on trypsin have a marked weakening action which may be easily shown by experiment.

d. But if the action of the pepsin and acid on trypsin is allowed to take place in the presence of a sufficient amount of protein the destructive effect is much less marked and a large part of the trypsin may be left little diminished in strength and capable of further action under proper conditions.

The explanation of these differences is simple enough. Protein is not the inert neutral substance the earlier physiologists assumed it to be but, because of its peculiar structure, may be very active in binding either acid or alkali. A gram of egg albumen or meat protein will hold 60 mg. or more of hydrochloric acid in amino acid salt combination. When protein is mixed with not more than 3.5 per cent. of its weight of acid in aqueous solution, pepsin present will have almost no effect in the way of digestion; with 6 per cent. the digestion is slow, while with 10 per cent. the digestion is rapid, provided the dilution is such as to not lower the hydrogen ion concentration too much. A certain value of the hydrogen concentration is necessary for the activation of pepsin; and if this does not obtain, protein will not be digested nor trypsin destroyed by the combination of acid and pepsin.

From this it would appear that trypsin administered by the mouth might readily persist in the stomach and pass through into the duodenum. Under certain limited conditions this is true, but a proper balance between acid and food protein would have to hold. In the absence of the right amount of protein the acid and pepsin would destroy much of the trypsin and the same thing would hold for the amylopsin.

With these facts in mind it must certainly appear irrational to administer the shotgun combination found in a number of mixed ferment preparations which

seem to be put together without much regard to the work supposed to be accomplished by them. I cannot agree with Mr. Beringer in his defense of the formulas of the National Formulary III.[1] In the Compound Elixir of Pepsin we have along with 10 gm. of pepsin, 1 gm. of pancreatin, 1 gm. of diastase (whatever that may be practically), and 1 c.c. of hydrochloric acid with approximately 360 mg. of the real acid. As the protein of the pepsin is often pretty fully saturated with acid this combination is such that the activity of the trypsin and amylopsin would be greatly impaired by the mixing operation. But worse than this is the small proportion of the pancreatin present. In the finished product there is a milligram to the cubic centimeter, while the dose is 8 c.c. This mixture may have some value as a pepsin product or as a vehicle, but the "compound" part of it is utterly absurd. Mr. Beringer complains that it was dropped from the National Formulary. It should never have been in there to begin with, because it is irrational. The Compound Powder of Pepsin is nearly as bad. While the dose of pancreatin provided for is greater, it is still too low to have therapeutic value, and the acid, not finding enough protein to bind it properly, would unquestionably weaken the ferments other than the pepsin on standing.

Mr. Beringer finds fault with the American Medical Association because it condemns such mixtures, which he seems to think should be retained since they were extensively prescribed. This is not a sound argument In the last two thousand years many things have been extensively prescribed which had no value whatever, and alas! this ignorant prescribing is still going on.

These mixtures, and similar ones, have been condemned because the amounts of trypsin or other pancreas ferment they contain are far too small to have any appreciable therapeutic value, even supposing the trypsin and amylopsin in them to remain active, which under the ordinary conditions of prescribing is extremely doubtful.

1. Jour. Am. Pharm. Assn., April, 1917.

Trypsin is indeed more stable in presence of acids than was formerly supposed, as several series of investigations from this laboratory have shown. But the practical conditions under which it can pass the stomach have to be carefully observed, and when administered at all this should be with the fewest possible complicating conditions. A few milligrams of trypsin can have at best but a vanishing effect. Large doses given at the right time may reasonably be expected to have therapeutic value, and prescribing should naturally have this end in view. As made at the present time, the pancreatins and their various combinations have no proper place in rational medicine, as proteolytic agents. There is here great room for improvement.

TYRAMIN AS AN ADJUNCT TO MORPHIN IN LABOR*

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NEW HAVEN, CONN.

(*From The Journal A. M. A., Sept. 15, 1917, p. 882*)

The employment of morphin in labor has become frequent in recent years, probably owing to the "twilight sleep" propaganda more than to any other influence. Briefly, morphin exhibits in this connection three important effects, the first desirable, the other two untoward, namely, analgesia, respiratory depression in the child, and delay of labor.

It is unnecessary to review in detail the interesting history of scopolamin-morphin anesthesia, including its rather haphazard introduction by Schneiderlin,[1] its development in obstetrics by the untiring enthusiasm of Gauss,[2] and its modification by the pharmacologic critique of Bürgi[3] (pantopon [pantopium hydrochloricum]), and of Straub[4] (a morphin-narcotin mixture, used by Siegel,[5] Libby[6] and others). Today its practice, so far as justified, is limited to a few who are fortunate enough to possess the combination of unusual facilities and almost superhuman patience.

Since Hatcher[7] seven years ago pointed out the almost complete lack of theoretical justification for the employment of scopolamin in this connection, little if

* The researches reported in this paper were encouraged by grants from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Schneiderlin: Aerztl. Mitt. a. und f. Baden, 1900, **54,** 101.
2. Gauss, C. J.: Arch. f. Gynäk., 1906, **78,** 579.
3. Bürgi, E.: Deutsch. Ztschr. f. Chir., 1913, **125,** 211-256.
4. Straub, W.: Biochem. Ztschr., 1912, **41,** 419.
5. Siegel, P. W.: München. med. Wchnschr., 1913, **60,** 2280.
6. Libby, W. E.: Scopolamin and Narcophin Seminarcosis During Labor, Jour. Am. Med. Assn., May 22, 1915, p. 1728.
7. Hatcher, R. A.: Scopolamin and Morphin in Narcosis and in Childbirth, Jour. Am. Med. Assn., Feb. 5, 1910, pp. 446-516.

any new evidence has been introduced in its favor, nor has our knowledge of its obscure pharmacology been very appreciably increased. This is due in part to unusual difficulties, especially the varied manifestations of scopolamin action, not only in different species but also in different individuals. The unstable character of the alkaloid has further increased the confusion.

In our laboratory, attempts have been made to throw more light on the relation of scopolamin to the three actions of morphin enumerated above.

To study the cerebral effect, Dr. J. F. Cobey has made a number of experiments on mice by the method of Fühner[8] and found evidence of some synergic action of the two drugs in this respect, supporting the results of Kochmann,[9] Bürgi[10] and their pupils. The respiratory effects of the scopolamin-morphin combination were tested by Dr. L. L. Maurer on an extensive series of rabbits, but we failed to convince ourselves that morphin depression of respiration is constantly affected by its combination with scopolamin. In a small number of cases the depression was delayed or preceded by acceleration.

Finally, as regards the action of scopolamin and morphin on the uterus itself, Mr. N. H. Copenhaver[11] and I were able to show no effect of either or both drugs on that organ, either isolated or in intact anesthetized animals, in doses at all comparable to those which are employed clinically. In unanesthetized animals, however, we[12] were able to show ultimately that small doses of morphin, by an effect definitely located in the cerebrum, tend to inhibit the rhythmic action of the uterus. This point has not been followed with respect to scopolamin.

8. Fühner, H.: Deutsch. med. Wchnschr., 1913, **39**, 143.

9. Kochmann, M.: Ztschr. f. exper. Path. u. Therap., 1913, **12**, 328.

10. Bürgi, E.: Deutsch. med. Wchnschr., 1910, **36**, 20.

11. Barbour, H. G., and Copenhaver, N. H.: Jour. Pharmacol. and Exper. Therap., 1915, **7**, 529, and Barbour, H. G.: Ibid., 1915, **7**, 547-555.

12. Barbour, H. G., and Copenhaver, N. H.: Proc. Soc. Exper. Biol. and Med., 1916, **13**, 159.

From the results noted above it will be seen that we could contribute no new theoretical considerations in support of the employment of scopolamin morphin anesthesia.

The value of morphin itself in labor has, however, been acknowledged by those best fitted to pass judgment. In view of this it would seem that a substance having actions more clearly understood and more desirable than those of scopolamin might be found which could be suitably combined with morphin. Particular effort should, of course, be directed to antagonizing the two most untoward effects of morphin.

The use in this connection of tyramin (para-hydroxy-phenyl-ethyl-amin hydrochlorid) or some related substance was suggested by the studies of Bry.[13] She pointed out the rather prolonged respiratory effect which tyramin and other phenylethylamin derivatives produce in mammals, and called attention to their oxytocic action which, however, had been noted by Dale and Dixon[14] in their studies of tyramin. Thus the two desired effects, respiratory and uterine stimulation, are exhibited by one substance.

Tyramin is an active principle of ergot, being derived by bacterial action on tyrosin-containing proteins. Its power to raise the blood pressure (associated with a close chemical relationship to epinephrin) has been made use of in therapy in connection with shock, for example by Clark[15] and Hoyt.[16] This point deserves more attention because of the advantages which this substance possesses over epinephrin in eliciting a more prolonged response and in lending itself readily to subcutaneous injection.

In seeking a morphin antagonist we first investigated a series of phenylethylamin derivatives in the hope of finding one even more suitable than tyramin. Dr. L. H. Nahum found, however, that tyramin hydrochlorid is but one tenth to one fifth as toxic as the hydrochlorids of phenylethylamin itself, and its sim-

13. Bry, G.: Ztschr. f. exper. Path. u. Therap., 1914, **16,** 186.
14. Dale, H. H., and Dixon, W. E.: Jour. Physiol., 1909, **39,** 25.
15. Clark, A.: Biochem. Jour., 1911, **5,** 236.
16. Hoyt, D. M.: Am. Jour. Med. Sc., 1912, **144,** 76.

pler benzyl derivatives (kindly prepared for us by Prof. T. B. Johnson of Yale).

Adopting tyramin, therefore, as the most promising substance available, Dr. Cobey studied its effect on morphin narcosis in mice, and concluded that any influence which it might have on the cerebral action of this drug was probably negligible.

Dr. Maurer[17] then investigated the respiratory volume and the condition of the respiratory center in rats subjected to various doses of tyramin and morphin, alone and combined. He was able to demonstrate very clearly an antagonism between the two substances which was best exhibited when they were administered in the ratio of approximately three parts of tyramin to one of morphin. Most significant was the observation that the respiratory action of the morphin often remained in abeyance during a period when marked analgesia was present.

We next transferred these studies to man, and have, in collaboration with Dr. W. C. von Glahn,[17] corroborated the antagonism in five normal individuals. We concluded that doses of from 40 to 50 mg. of tyramin administered simultaneously with a therapeutic dose of morphin (16 mg.) will completely antagonize the depressant action of morphin on the respiration. The respiratory stimulation produced by tyramin is probably indirect and due to an augmentation of oxidative processes, for the drug has a tendency to increase the respiratory exchange.

The uterine action of tyramin, which has been elicited in laboratory animals by Dale and Dixon,[14] Guggenheim[18] and others, was first demonstrated on the excised human uterus by C. C. Lieb.[19] Hitherto it has not been satisfactorily investigated in the obstetric clinic. Heimann,[20] Zimmermann,[21] Jäger[22] and Krosz[23]

17. Barbour, H. G.; Maurer, L. L., and von Glahn, W. C.: Jour. Pharmacol. and Exper. Therap., 1916, **8**, 124.
18. Guggenheim, M.: Therap. Monatsh., 1912, **26**, 795.
19. Lieb, C. C.: Am. Jour. Obst., 1915, **71**, 209.
20. Heimann: München. med. Wchnschr., 1912, **59**, 1370.
21. Zimmermann, R.: München. med. Wchnschr., 1913, **60**, 2675.
22. Jäger, F.: München. med. Wchnschr., 1913, **60**, 1714.
23. Krosz: Zentralbl. f. Gynäk., 1913, **37**, 1507.

all employed doses which were far too small (maximum 2 mg.) to be effective. In the last three cases the issue is confused by the combination of tyramin with histamin. Even Kehrer's[24] employment of from 10 to 20 mg. intramuscularly, and Sharp's[25] use of 30 mg. subcutaneously may scarcely be considered a fair test.

The employment of tyramin-Roche in effective doses as an adjunct to morphin is now under investigation by Dr. C. L. Deming on carefully selected cases of normal labor in the obstetric clinic of Dr. J. M. Slemons at Yale. The number of cases thus far has been too few to justify broad statements. It may, however, be said that the laboratory data are being well corroborated. The dosage employed is 16 mg. of morphin sulphate given hypodermically in solution with 40 mg. of tyramin. In the absence of contraindications, this injection is given when discomfort becomes marked in the first stage of labor. Analgesia appears to be as complete as though the same dose of morphin were given alone.

The respiratory rate of the mother becomes slightly increased rather than decreased, and usually remains somewhat accelerated throughout. The condition of the children has been quite satisfactory, no tendency to asphyxia having been observed. In every case the frequency of the uterine contractions has been increased within five minutes after the injection, and this augmented activity maintained throughout. The increase has usually been from five minute intervals to intervals of about two minutes with an augmentation, temporarily at least, in the strength of individual contractions. Forty mg. of tyramin produce a temporary rise in blood pressure usually amounting to 20 to 25 mm.; this seems to be negligible in normal cases, but should be borne in mind and followed closely.

It is hoped that more complete data can soon be offered, but there seems in the meanwhile no objection to the employment of tyramin and morphin by those obstetricians, and only those, who are thoroughly versed in the use of morphin in labor.

24. Kehrer, E.: München. med. Wchnschr., 1912, **59**, 1831.
25. Sharp, J. G.: Proc. Roy. Soc. Med., 1911, **4**, 114.

OILED GAUZE AND THE ABSORBING POWER OF COTTON SPONGES *

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(From The Journal A. M. A., Sept. 29, 1917, p. 1073)

"Nonadhering surgical gauze" was introduced by H. E. Fisher.[1] It was prepared by saturating the gauze with a soft paraffin mixture made by the addition of petrolatum, lanolin or stearic acid to paraffin. Fisher asserts especially that the blocking of the fibers prevents matting with secretions and débris; that it prevents adherence of the gauze, and that the granulations of tissue repair are not injured when the dressing is removed.

While working on paraffin bandages, I became interested in the permeability of such bandages as influenced by various waxes and oily preparations. A series of gauzes of loose and close mesh were prepared by impregnating them with paraffins of different hardness, ranging from hard paraffin to liquid petrolatum.

As the result of experiments I find that "oiled gauze," that is, gauze that is impregnated with liquid petrolatum, holds out considerable promise of usefulness. Cotton sponges wrapped in this oiled gauze absorb viscid fluids very much better than when wrapped in plain gauze; the gauze is soft and pliable; it is easily prepared, and it can be sterilized by heat after impregnation.

In the course of the investigation a loose mesh cheesecloth and a close mesh muslin were compared; it was found that the former permits much better absorption. I also used a series of mixtures of

* From the Pharmacologic Laboratory of the Western Reserve University, School of Medicine.

* Partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Fisher, H. E.: Nonadhering Surgical Gauze, The Journal A. M. A., March 25, 1916, p. 939.

paraffins[2] for impregnating the gauzes, but found that liquid petrolatum alone is superior. This oiled gauze is prepared by dipping the cloth into the liquid petrolatum and expressing out the excess.

The technic of making the absorption tests was as follows:

Sponges were prepared by wrapping 1 gm. of absorbent cotton in a piece of the gauze, 12 cm. square. Egg white and egg yolk were used to simulate wound discharges. The egg white or egg yolk was placed in a flat bottom pan, in a layer perhaps 2 to 3 mm. thick; and in this were placed the sponges, which had previously been weighed. The sponges were reweighed at intervals. The results are shown in detail in the chart.

The results of the experiments may be thus summarized:

1. Sponges made of compressed cotton[3] absorb better than those made of loose cotton.

2. Sponges made with the cotton layers parallel to the surface absorb much better than those made with the layers vertical to the surface.

3. The sponges wrapped in loose mesh fabric absorb somewhat better than those wrapped in close mesh fabric.

4. Sponges filled with cotton absorb much better than those filled with powdered charcoal.

5. Sponges covered with gauze impregnated with liquid petrolatum absorb very much better than sponges covered with plain gauze.

6. Sponges covered with gauze impregnated with 50 per cent. liquid petrolatum are intermediate.

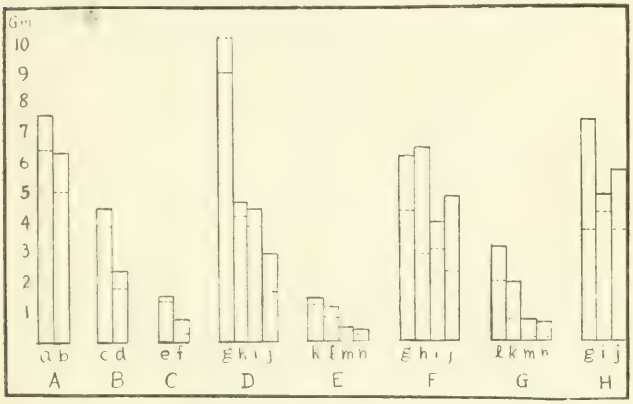
7. Sponges covered with gauze impregnated with Fisher's mixture do not absorb as well even as those covered with plain gauze.

2. The following paraffins and oils were employed: hard paraffin (Parowax brand); 20 per cent. white petrolatum: paraffin (Parowax), 80 parts, white petrolatum, 20 parts (Fisher's formula); 1 per cent. naval pitch: preceding mixture with 1 per cent. naval pitch added; 50 per cent. liquid petrolatum; equal parts of paraffin (Parowax) and liquid petrolatum; liquid petrolatum (Stanolind liquid paraffin).

3. The finished sponge was compressed in an old style letter press.

8. The influence of oils, etc., on absorption is practically the same for loose mesh as close mesh gauze; and for egg albumin and egg yolk.

The favorable influence of the oiled gauze is explained by the protection that the oil furnishes against swelling of the thread and obstruction of the mesh.



Absorption of fluid by sponges: The dotted line shows the grams of fluid absorbed in one hour, and the solid line that absorbed in twenty-four hours. *A* to *E* represent the absorption of undiluted egg white; *F* and *G* that of undiluted egg yolk, and *H* that of beaten egg. *A*, comparison of (*a*) compressed and (*e*) plain cotton, wrapped in cheesecloth; *B*, comparison of cotton applied (*c*) horizontaly and (*d*) vertically; *C*, comparison of (*e*) cotton in muslin and (*f*) charcoal in muslin; the cotton, as in all these experiments, weighed 1 gm.; the charcoal pad contained 4 gm. of granular charcoal; *D* to *H*, comparison of treated and untreated gauze; (*g*) Stanolind cheesecloth; (*h*) 50 per cent. Stanolind cheesecloth; (*i*) plain cheesecloth; (*j*) Fisher cheesecloth; (*k*) plain muslin; (*l*) Stanolind muslin; (*m*) 50 per cent. Stanolind muslin; (*n*) Fisher muslin.

I have not yet had the opportunity of investigating whether it is as little adhesive as the Fisher preparations; but it is certainly more favorable to absorption, and I would therefore suggest its use for dressing moist wounds.

THE PREVENTION OF SIMPLE GOITER IN MAN

A SURVEY OF THE INCIDENCE AND TYPES OF THYROID ENLARGEMENTS IN THE SCHOOLGIRLS OF AKRON (OHIO), FROM THE 5TH TO 12TH GRADES, INCLUSIVE — THE PLAN OF PREVENTION PROPOSED *

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(*From The Journal of Laboratory and Clinical Medicine, October, 1917*)

Simple goiter in animals is probably the easiest of all known diseases to prevent. Simple goiter includes all the thyroid enlargements seen in the lower animals and those thyroid enlargements seen in man, except cases properly classified as exophthalmic goiter. Many cases with simple goiter later develop exophthalmic goiter. In brief, simple goiter includes all those thyroid enlargements formerly classified as endemic, epidemic and sporadic. The periods when it most frequently develops are (1) fetal, (2) adolescent, and (3) during pregnancy. Anatomically, a wide range of changes may be present, depending on the species of animal and on the stage (duration) of the disease. In man and fowls one more commonly sees the form characterized by an abundance of colloid material — the so-called "cystic or colloid goiter" of older writers, while in goiter of dogs, sheep, cattle, pigs, fish, etc., the accumulation of colloid material is seen only in the late, regressive or quiescent stages. Again in man

* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

* Aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

the adenomatous form is very common and is exceedingly rare if present at all in the lower animals.

It will not be possible to review all the experimental data on which the assertion, that simple goiter in animals is an easily preventable disease, is based. Certain of the more important facts bearing on the subject will be summarized as an introduction to the discussion of the means proposed to attempt the prevention of simple goiter in man.

1. The developmental stage of all goiters is characterized by an increased blood flow, an increase in the size and number of epithelial cells, a decrease in the stainable colloid of the follicular spaces and a marked absolute decrease in the iodine content. The decrease in iodine precedes the cellular changes.

2. Similar thyroid changes (compensatory hyperplasia) invariably occur in the remaining portion of the gland when a sufficient portion of the entire gland is removed. The amount of gland it is necessary to remove in order to cause compensatory hyperplasia varies somewhat with the species of animal, definitely with the age, the diet, and the presence of iodine.

3. The administration of exceedingly small amounts of any salt of iodine thus far tried in any manner completely protects the remaining thyroid against compensatory hyperplasia, even after the removal of three fourths of the normal gland in cats, dogs, rabbits and rats, fowls and pigeons. Halsted[1] and Hunnicutt[2] reported a series of partial thyroidectomies in dogs in which they failed to obtain the hypertrophy or hyperplasia of the remaining portion and, therefore, concluded that Halsted's earlier and justly classic experiments[3] on the production of compensatory hyperplasia by partial removal were not due to thyroid removal,

1. Halsted, W. S.: Reconsideration of the Question of Experimental Hypertrophy of the Thyroid Gland, and the Effect of Excision of This Organ Upon Other Ductless Glands, Am. Jour. Med. Sc., 1914, **148**, 56.

2. Hunnicutt: Absence of Hyperplasia of Remainder of Thyroid in Dog After Piecemeal Removal of This Gland. Autotransplantation of Thyroid in Partially Thyroidectomized Animals, Am. Jour. Med. Sc., 1914, **148**, 207.

3. Halsted, W. S.: Experimental Study of Thyroids of Dogs, Johns Hopkins Hosp. Report, 1896, **1**, 373.

but to something else, possibly infection. Their failure to obtain compensatory hyperplasia in the second series was really due to the presence of available iodine either from the absorption of iodine painted on the skin or from contact with other dogs, or from inhalation of volatilized iodine from other dogs carrying iodine, or from other sources in the rooms.

4. If most of the thyroid gland is removed before or in the early stages of pregnancy and rigid steps are taken to exclude available iodine, the pups at birth will have enlarged thyroids, as first shown by Halsted,[3] while if available iodine is present, the pups will have normal thyroids.[4]

5. We have repeatedly found that a milligram of iodine given at weekly intervals is sufficient to prevent thyroid enlargement, although other pups of the same litter, living in the same kennel, and eating the same food, regularly developed goiter.

6. The thyroid gland has an extraordinary affinity for iodine, as can readily be shown by perfusion experiments in vitro or by injecting small amounts — 5 to 20 mg. KI — into the circulation.[5, 6] Experimentally, then, the proof is sufficiently complete to demonstrate the underlying principles of goiter prevention in animals and the ease with which they can be applied. From the practical standpoint, the first instance of preventing goiter on a large scale was accidental and in connection with the sheep raising industry of Michigan. Prior to the discovery of salt deposits around the Great Lakes, the future of the industry seemed hopeless, but with the development of the salt industry and its use by the sheep growers, goiter rapidly decreased. The salt contains appreciable quantities of

4. Marine and Lenhart: Effects of the Administration or the Withholding of Iodin-Containing Compounds in Normal, Colloid, or Actively Hyperplastic Thyroids of Dogs. Some Experiments on Prenatal Thyroid Hyperplasia in Dogs, etc., Arch. Int. Med., 1904, **4**, 253.

5. Marine and Feiss: The Absorption of Potassium Iodid by Perfused Thyroid Glands and Some of the Factors Modifying It, Jour. Pharm. and Exper. Therap., 1915, **7**, 557.

6. Marine and Rogoff: The Absorption of Potassium Iodid by the Thyroid Gland in Vivo Following Its Intravenous Injection in Constant Amounts, Jour. Pharm. and Exper. Therap., 1916, **8**, 439.

both bromine and iodine, and in places these elements are extracted on a commercial scale. The second instance of goiter prevention on a large scale was in brook trout. Some years ago the development of goiter in artificially raised members of the salmon family became alarming, and many plants were abandoned on account of the disease. After considerable work, which led to the conclusion that the disease was simple goiter, we were able to completely prevent the disease in several hatcheries, by the use of very small amounts of tincture of iodine added to the water.[7] Later the attempt was made to substitute whole sea fish for part or all of the diet, which, likewise, proved to be, from the practical point of view, a cheaper and simpler method of complete prevention.[8] Similar preventive work with farm stock is being carried out under our direction in some of the valleys of British Columbia, where goiter was so prevalent that farmers were unable to raise hogs, cattle, horses and chickens on account of myxedema (cretinism). Similar work in the prevention of goiter in hogs was recently reported by Smith.[9] He was able to completely prevent fetal myxedema by the use of potassium iodide to the mother during pregnancy. He, however, used quantities far in excess of those necessary to prevent goiter and myxedema. In spite of this knowledge of the ease and simplicity of goiter prevention in the lower animals, we know of no instance where the attempts has been made to systematically prevent or control the disease in children in large communities, especially those of the Great Lakes Basin, where goiter is so prevalent. Locally, we have been carrying out preventive treatment for the past six years at the Lakeside Hospital Medical Dispensary and have urged local physicians to do so in their private practices. A

7. Marine and Lenhart: Observations and Experiments on the So-Called Thyroid Carcinoma of Brook Trout (Salvelinus Fontinalis) and Its Relation to Ordinary Goiter, Jour. Exper. Med., 1910, **12,** 311.

8. Marine: Further Observations and Experiments on Goiter (So-called Thyroid Carcinoma) in Brook Trout (Salvelinus Fontinalis). III Its Prevention and Cure, Jour. Exper. Med., 1914, **19,** 70.

9. Smith, G. E.: Fetal Athyreosis. A Study of the Iodin Requirements of the Pregnant Sow, Jour. Biol. Chem., 1917, **29,** 215.

great deal has been accomplished in this way, but as it is a public health matter the most practical and economic method would be to utilize the public school system and the board of health. When the medical inspection of schools is more or less independent of the board of health, it would be carried out through the medical director of schools. This year it has been possible to begin such work on a large scale in the city of Akron, through the cooperation of the superintendent of schools, the board of education and the county medical society.

It was decided for the present to limit the prophylactic work to the girl pupils, since adolescence is the most important goiter developing period, and since at this period it occurs about six times more frequently in girls than in boys.

The plan now in operation was arranged from the standpoint of simplicity, practicability, economy, and the possible scientific value of the data obtained. Changes will doubtless be made as the work progresses. First a census of the condition of the thyroid gland was taken of all girls between the 5th and 12th grades inclusive, and the findings recorded on individual cards, of which the following is a copy:

No.	Date
Name	School
Age Weight	Physical Development
Grade	Class Standing
Tonsils-Adenoids	
Thyroid	1
Simple	2
Adenomas	3
Thyroid-tract	4
Duration	
Remarks	

The thyroid examinations of all pupils were made by a single examiner in order to make the standards used constant and the data obtained uniform. It is planned to take the census each year in the same way.

For the prophylactic treatment we have selected sodium iodide on the grounds of economy and ease of administration. Regarding the amounts that should be given, we have no data except those from animal experimentation. As has been pointed out repeatedly, exceedingly small amounts of iodine are needed. One milligram of iodine given weekly, by mouth, is ample to prevent goiter in dogs. In all our dispensary experiments with children we have used either syrup of hydriodic acid or syrup of ferrous iodide, in 1 c.c. doses, daily for two to three weeks, repeated twice yearly, and have recommended their use to clinicians solely because they were the only U. S. P. preparations sufficiently dilute to offset the tendency to use too large amounts.

We have, therefore, arbitrarily selected to use 2 gm. sodium iodid, given in 0.2 gm. doses each school day, for each pupil in the 5th, 6th, 7th and 8th grades; and 4 gm. given in 0.4 gm. doses each school day for each pupil in the 9th, 10th, 11th and 12th grades. These amounts will be given twice annually about the first of May and December, at the schools by the teachers or nurses. Bottles were distributed to the several schools, containing the solutions (0.2 gm. NaI in 5 c.c. H_2O and 0.4 gm. in 5 c.c. H_2O) in sufficient amounts to give each pupil electing to take the prophylactic treatment a total of 50 c.c. A record was made both of those who took the treatment and of those who did not. All pupils will be examined annually and the thyroid conditions recorded. These amounts of sodium iodide provide approximately 1,700 (1,692) mg. of iodine for each pupil of the 5th, 6th, 7th and 8th grades, and approximately 3,400 (3,384) mg. for the 9th, 10th, 11th and 12th grades. When one recalls that 25 to 30 mg. saturates the normal thyroid of 20 to 25 gm. and that the thyroid has an extraordinary affinity for iodine, it seems like a prodigious waste, and we believe it is. The amounts used at the start were purposely made excessive to provide for any unknown factors and will probably be materially reduced.

Analysis of the Thyroid Examinations. — Three thousand eight hundred and seventy-two girls of the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th grades were examined and the general result is given in the following tabulation.

TABLE 1. CONDITION OF THYROID GLAND

	Normal	Slight Enlargement	Moderate Enlargement	Marked Enlargement	Adenomas	Thyroid Tract (Persistent)
Total	1,688	1,931	246	7	39	504
Per cent.	43.59	49.88	6.35	0.18	1.01	13.4

The thyroid glands were examined from the standpoint of *normals, slight, moderate* and *marked enlargements, adenomas, persistent thyroglossal tracts* and the pupils for gross manifestations of *myxedema*, and *exophthalmic goiter*. No obvious case of either myxedema or exophthalmic goiter was found.

Under *normal* we have included all glands (*a*) which are not visible as a bulging of the skin across the trachea, (*b*) having a barely detectable band of thyroid tissue across the trachea on palpation and (*c*) absence of well defined thyroglossal stalk (so-called pyramidal process).

Those cases with enlarged thyroids have been divided into three arbitrary groups (1) *slight*, (2) *moderate* and (3) *marked* enlargement. Under *slight enlargement* we have grouped those cases with (*a*) visible bulging of the skin over the thyroid isthmus (except in the very stout children) and (*b*) a widened and thickened isthmial band or mass on palpation. If the isthmus cannot be seen or felt, it can be felt by having the child swallow, while the finger or thumb is held against the trachea just below the cricoid cartilage.

Under *moderate enlargement* we have grouped those with gross deformity — bulging of the neck laterally from the enlarged lobes and marked bulging of the skin anteriorly from the enlarged isthmus. In approximately 93 per cent. the right lobe was larger than the left, which is about the usual percentage.

Under *marked enlargement* we have grouped those cases with excessive deformity. One thousand six hundred and eighty-eight, or 43.59 per cent. of all pupils examined, were classed as normal; 1,931, or 49.88 per cent., were classed as slightly enlarged; 246, or 6.35 per cent., were classed as moderately enlarged (none of which had been operated upon); 7, or 0.18 per cent., were classed as markedly enlarged, of which two had been operated upon. This gives as totals, 2,184, or 56.41 per cent., with enlarged thyroids, and 1,688, or 43.59 per cent., with normal thyroids. In 39 cases, or 1.01 per cent., adenomas, single or multiple, were detected. The smallest was approximately 2 cm. in diameter and the largest about 6 cm. These figures are of little value, since they include only the large superficial and favorably located ones.

The thyroglossal tract when present is very readily detected, either slightly to the right or left of, and rarely in, the midline. Only those which extended to the base of the thyroid cartilage were included. In many it was palpable to the hyoid bone. The very small pyramidal processes ending below the cricoid cartilage were not included. Five hundred ninety-four, or 13.4 per cent. of the cases, had well defined thyroid stalks. Physiologically, the presence of thyroid tissue in the line of descent of the embryologic thyroid anlage indicates that the gland had undergone enlargement in intrauterine life, whereas normally the tract undergoes absorption beginning, according to His,[10] in the second month. The presence of large amounts of thyroid tissue about the foramen cecum — the so-called lingual thyroid — or of large masses between the hyoid bone and thyroid cartilage — so-called infrahyoid thyroids — are of the same significance. Excluding the rare congenital defects in the thyroid anlage, the amount of thyroid tissue in the line of descent of the thyroid gland may be used to determine the degree of normality of the thyroid gland in intrauterine life, and as first pointed out by Strec-

10. His, W.: Der Tractus Thyreoglossus und Seine Beziehungen zum Zungbein, Arch. f. Anat. u. Physiol. (Anat. Abteilung), 1891, 26-32.

keisen,[11] it is an excellent index for determining the extent and degree to which a given district is affected with simple goiter. At Basel he found about 79 per cent. of the cases coming to postmortem examination had persistent thyroglossal stalks. If the district is

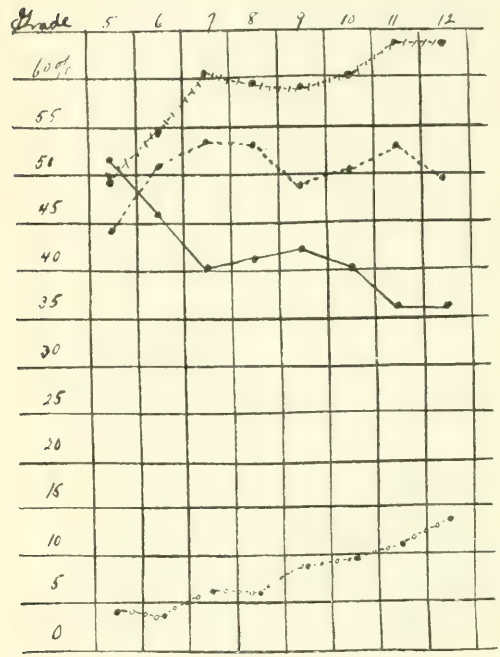

Fig. 1.

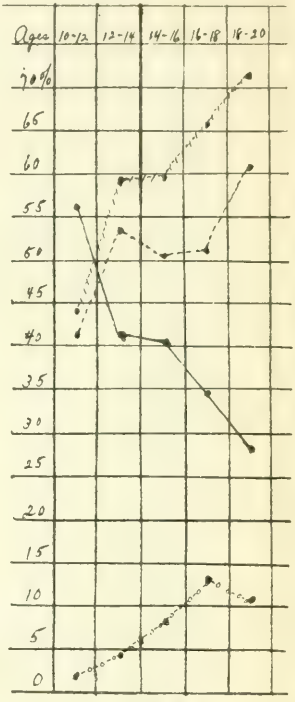

Fig. 2.

——— normals; - - - - - slight enlargements; -o-o-o-o moderate enlargements; -/-/-/-/- total enlargements.

extremely goitrous and the mothers are not fed iodine during pregnancy, practically all children should have large persistent thyroglossal tracts. If the district is nongoitrous (e. g., sea cost regions) very few children have persistent thyroglossal tracts.

11. Streckeisen: Beiträge zur Morphologie der Schilddrüse, Virchows Arch. f. path. Anat., 1886, **13**, 131, 215.

TABLE 2. CONDITION OF THYROID ARRANGED BY GRADES

	Grade 5		Grade 6		Grade 7		Grade 8		Grade 9		Grade 10		Grade 11		Grade 12	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	410	51.77	354	45.90	269	40.0	206	40.47	191	42.92	124	40.00	76	36.02	58	36.02
Slightly enlarged	350	44.20	388	50.33	360	53.49	271	53.24	215	48.31	155	50.00	112	53.08	80	49.70
Moderately enlarged	31	3.90	29	3.76	43	6.39	31	6.09	28	8.54	30	9.68	23	10.90	21	13.04
Markedly enlarged	1	0.13			1	0.14	1	0.20	1	0.23	1	0.32			2	1.24
Totals	792	20.45*	771	19.94	673	17.38	509	13.15	445	11.5	310	8.00	211	5.45	161	4.16
Adenomas†	3	0.13	3	0.13	7	0.52	6	0.28	8	0.36	6	0.28	5	0.22	1	0.04

* Percentage of total pupils examined, 3,872.
† Adenoma percentage figured from the total enlarged thyroids, 2,484.

TABLE 3. CONDITION OF THYROID ARRANGED ACCORDING TO AGES

	Age 10-12		Age 12-14		Age 14-16		Age 16-18		Age 18-20	
	No of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	530	56.08	521	41.32	460	40.35	156	34.41	21	28.77
Slightly enlarged	394	41.69	680	53.92	578	50.70	235	51.88	44	60.27
Moderately enlarged	21	2.22	59	4.68	98	8.6	60	13.24	8	10.96
Markedly enlarged	.....	.....	1	0.08	4	0.35	2	0.44		
Totals	945	24.41*	1,261	32.56	1,140	29.44	453	11.70	73	1.89
Adenomas	2	0.01†	11	0.52	18	0.84	8	0.39		

* Percentage of total pupils.

† Percentage of total enlarged thyroids.

Following the analysis further, the condition of the thyroid in relation to grades is shown in Table II and the accompanying curve chart (Fig. 1); and in relation to age, in Table III and accompanying curve chart (Fig. 2).

The most rapid increase in the number of slight enlargements occurs between the 5th and 8th grades. This corresponds very closely with the rapid increase, between the 10th and 14th years. The average age of the 5th grade pupils was 10 years. Less than 2 per cent. of the 5th grade were under 10 years and they were tabulated in the 10 to 12 age group.

The age group 18 to 20 contains less than 2 per cent. of the total pupils, and while tabulated for the sake of completeness, the percentages are doubtless higher than the normal average for this age and properly belong to a special group with lower mental activity. The relation of thyroid enlargement to retarded mental development is an important subject, but our available data do not permit of further discussion at present.

DISCUSSION

The most valuable and accurate data of the incidence of goiter in America can be obtained from examinations of the public school population, because, in the first place it covers the most important ages when goiter develops; secondly, it gives the most complete census; and thirdly, no additional expense or additional effort is necessary. Up to the present time no organized and systematic effort has been made in this country to study the incidence of goiter in the school populations of large communities, even in the Great Lakes Basin — the largest and most densely populated of all goiter districts of North America.

The report by Hall[12] of the examination of 3,339 students at the University of Washington is the most extensive available in American literature. Of the 2,086 men with the average age of 20 years and 5

12. Hall, D.: The Prevalence of Goiter in the Northwest, Based on the Examinations of 3339 Students Entering the University of Washington, Northwest Medicine, 1914, **6**, 189, 371.

months, he found 374, or 17.93 per cent., with enlarged thyroids; 272, or 13.03 per cent., classed as perceptible; 92, or 4.43 per cent., classed as medium, and 10, or 0.48 per cent., classed as large. Of the 1,253 women, with the average age of 19 years and 3 months, he found 388, or 30.98 per cent., with enlarged thyroids; 294, or 23.45 per cent., classified as perceptible; 85, or 6.79 per cent., classified as medium, and 9, or 0.7 per cent., classified as large. These figures demonstrate clearly the prevalence of goiter in the northwestern states. The group is too selective and the ages too advanced to give an average incidence percentage, because (*a*) the greatest incidence occurs during puberty, (*b*) a certain percentage of enlargements recede below the level of clinical detectability spontaneously and (*c*) a small percentage would have receded because of iodine feeding.

In the Great Lakes Basin, Olsen[13] reports the examination of 606 women and 193 men, presumably between the ages of 18 and 60, at Chicago. Among the women, he found an average of 17.87 per cent. affected and among the men 6.72 per cent. The figures emphasize the frequency of thyroid enlargements, though they are very much lower than would be obtained from a similar number of examinations during the school age, on account of the factors of spontaneous or induced regression of the thyroid enlargements and of migrations from nongoitrous districts, which his figures necessarily include.

In Europe the statistics of Schittenhelm and Weichardt[14] deal with the incidence of goiter in the school populations of certain districts of Bavaria, where goiter is prevalent. Using very liberal standards, they report incidences as high as 77 and 89 per cent. of the school population affected.

13. Olsen, E. T.: Goiter, Its Prevalence in Chicago, as Shown by the Examination of 800 Individuals, Illinois Med. Jour., Springfield, 1915, **27**, 16.

14. Schittenhelm, A., and Weichardt, W.: Der Endemische Kropf mit Besonderer Berücksichtigung des Vorkommens im Königreich Bayern, J. Spreuger, Berlin, 1912.

In the Vosges mountains of eastern France and Alsace, MacAuliffe[15] has recently reported the examination of 2,311 children between the ages of 2 and 15 years. He found 288, or 12.5 per cent., affected. A comparison of our data with the data cited above is not possible. We use a much more rigid standard of normal, both clinically and anatomically. Anatomically, the strictly normal gland does not exceed 0.5 gm. thyroid per kilogram of body weight, though many European writers, especially those in the Alpine goiter districts, allow as much as 1.0 gm. per kilogram. In dogs, the normal thyroid gland does not exceed 0.3 gm. per kilogram. Clinically, in the normal gland the isthmus can barely be felt, but the lateral lobes cannot be felt.

The question of the production of exophthalmic goiter by the use of iodine may be mentioned briefly. Some Swiss writers, like Oswald,[16] take the extreme view that iodine should never be used in goiter, because of the danger of producing exophthalmic goiter. Pineles[17] and Kocher[18] take the more moderate ground that iodine should be given cautiously to neurotic individuals with goiter. Our experience has led us to the conclusion that the risk of inducing manifestations of exophthalmic goiter from the use of iodine in physiologic doses is exceedingly small, even in those cases with large hyperplastic thyroids; i. e., the kind of thyroid enlargement which would permit of the most rapid formation and excretion of the iodine-containing hormone. The extent to which iodides are used in general medicine and surgery and the rarity of the development of signs of exophthalmic goiter is the best index of the danger. Iodine is usually employed in immensely large doses; 0.2 to 0.4 gm. NaI daily for two weeks would offer a great excess over the amounts necessary to saturate even the largest thyroids and probably much smaller amounts would

15. MacAuliffe, L.: Goitre, cretinisme et myxedéme dans les Hautes-Vosges, Bull. de l'Acad. de méd., Paris, **75**, 127.

16. Oswald: Die Gefahren der jodbehandlung, Corresp. Bl. f. schweiz. Aerzte, 1915, **45**, 641.

17. Pineles: Ueber die Empfindlichkeit des Kropfes gegen Jod, Wien. klin. Wchnschr., 1910, **23**, 353.

18. Kocher: Ueber jod Basedow, Arch. f. klin. Chir., 1910, **92**, 1166.

suffice in man, as it has been proved to do in the lower animals.

While the danger of causing symptoms of exophthalmic goiter probably varies with the size and degree of active hyperplasia, all authors agree that the important factor in determining such symptoms lies outside the thyroid, either in the nervous system, or some gland like the adrenal, and antedates any thyroid changes. Klose[19] has reported the production of exophthalmic goiter in nervous fox terrior dogs, by the injection of sodium or potassium iodide in 0.6 gm. doses per kilogram. Those experiments were soon discredited by the work of Bordenhewer.[20] No one else has suggested any danger from the use of iodides in the case of nongoitrous individuals, except the well-known acute iodism, which affects a small percentage of people, and, so far as known, is not related to thyroid activity. Cases with definite manifestations of exophthalmic goiter should not be given iodine, although there are cases (or better, stages) of the disease which are distinctly benefited by iodides.

The use of desiccated thyroid has well known dangers after adolescence — mainly because of the large doses used. Both economically and practically, it would not be suitable for general use, as a prophylactic agent.

SUMMARY

In a complete census of the condition of the thyroid gland in the girls from the 5th to 12th grades of the school population of a large community in the Great Lakes goiter district, it was found that 1,688, or 43.59 per cent., had normal thyroids; 2,184, or 56.41 per cent., had enlarged thyroids, and 594, or 13.4 per cent., had well defined, persistent thyroglossal stalks. The community lies near the southern edge of the goiter district and it is suggested that communities near the lakes would show a higher incidence. The method of prophylaxis proposed is in operation.

19. Klose: Experimentele Untersuchungen über die Basedow'sche Krankheit, Arch. f. klin. Chir., 1911, **95**, 649.

20. Bordenhewer: Erzeugt Iodeinspritzung Morbus Basedow? Arch. f. klin. Chir., 1912, **97**, 729.

COMPARATIVE ACTIVITY OF LOCAL ANESTHETICS: I. PARALYSIS OF MOTOR NERVE FIBERS

DIRECT APPLICATION TO THE SCIATIC NERVE OF THE FROG *

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(From The Journal of Pharmacology and Experimental Therapeutics, November, 1917)

I. INTRODUCTION

The discovery of the numerous local anesthetics has naturally led to the wish to select "the best." Many attempts have been made to do this by laboratory methods. A review of the numerous papers show considerable discrepancies—some perhaps due to imperfect experimentation, many to failure to realize the limitations of a particular method, and therefore to the drawing of conclusions that the experiments do not justify. The clinical conclusions appear open to the same questions.

In view of the great importance of the subject, it seems worth while to reopen the more important and promising lines of investigation. I have therefore planned to compare those anesthetics that appear most

* This investigation was supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

useful in practice, or that have some other special interest; and to investigate these by methods and under such conditions as may be expected to throw a light on their specific advantages in the various fields in which local anesthetics are employed.

The clinical methods of the application of local anesthetics may be reduced for the present purposes to three direct types:

1. *Conduction anesthesia,* i. e., application to nerve trunks, peripherally and in the spinal canal. This is reproduced experimentally by immersion of the nerve trunk in the solution. It involves the direct action of the anesthetic agent on the nerve fibers, with the minimum of complicating factors. The motor fibers have been used exclusively for these tests, because of technical convenience. It is possible, however, to extend the method to the sensory fibers, and thus to approach more closely to the clinical problem.

2. *Terminal anesthesia,* i. e., application to the smaller fibers in the distribution of the nerve. This is used clinically in the infiltration method. The same principles apply also to the hypodermic use. In these methods, the clinical efficiency depends not only on the action of the nerve fibers, but is complicated by the rate of absorption of the drug from the field of operation. Experimentally, this method is reproduced by the "wheal (quaddel) method" of intracutaneous injection.

3. *Surface anesthesia,* i. e., the application to superficial surfaces, especially mucous membranes (eye, nose, etc.). The efficiency here involves penetration through the membrane, as well as the action on the nerves and the removal by absorption into the tissues. Experimentally, the method is reproduced by the application to the cornea, or to the frog's foot.

The paralysis of the motor fibers by direct application to the sciatic nerve of frogs was selected for the first paper, because it is the simplest method and promises to give the most exact results. It would therefore be most likely to elucidate the principles. Its

results, however, should not be transferred directly to the clinic, until they have been checked by other methods.

Critic of the Motor-Fiber Method.—The immersion of the nerve-trunk directly in the anesthetic solution reduces accidental disturbances to the minimum. This method variously modified has therefore been used extensively, whenever exact comparisons were desired (Mommsen,[1] 1881; Laewen,[2] 1906; Gros,[3] 1910, 1912; Symes and Veley,[4] 1911; Hoffmann and Kochmann,[5] 1912; Zorn,[6] 1913; Closson,[7] 1914). The sciatic nerve of the frog is usually employed.

Several possible fallacies have, however, been generally recognized: 1. In principle, it must not be overlooked that the method measures paralysis of the motor fibers. It is an open question as to how far it is justifiable to transfer these results to sensory fibers and nerves. It is known that sensory fibers are generally more easily paralyzed than motor fibers. It is not known whether the ratio of motor paralysis to sensory paralysis is the same for all anesthetics. Stovain, at least, is a departure from this rule (Santesson, quoted by Fromherz,[8] 1914). The question can only be answered by experimentation.

2. It must not be forgotten that experiments on exposed nerves cannot be transferred directly to injections or applications to mucous membranes, since the conditions of absorption are not reproduced.

3. The criteria of paralysis are arbitrary and have been applied differently by different investigators.

1. Mommsen, J.: Virch., 1881, **83,** 243.

2. Laewen, A.: Beitr. z. klin. Chir., 1906, **50,** 621; Arch. f. exper. Path. u. Pharmakol., 1906, **56,** 138.

3. Gros, O.: Arch. f. exper. Path. u. Pharmakol., 1910, **62,** 380; ibid., **63,** 80; ibid., 1912, **67,** 127, 132.

4. Symes, W. L., and Veley: Proc. Roy. Soc., B, 1911, **83,** 421.

5. Hoffmann, A., and Kochmann: Deutsch. med. Wchnschr., 1912, **48,** 2264.

6. Zorn, L.: Ztschr. f. exper. Path. u. Therap., 1913, **12,** 529.

7. Closson, O. E.: Jour. Michigan Med. Soc., 1914, **13,** 587.

8. Fromherz, K.: Arch. f. exper. Path. u. Pharmakol., 1914, **76,** 257.

This makes it impossible to compare their absolute values, although the ratios should presumably be constant.

The absence of motor response to sciatic stimulation depends not only on the concentration of the solution and on the time of immersion, but also on the length of the immersed segment of nerve, on the size of the nerve, on the distance between the muscle and the stimulated point of the nerve, on the temperature, on the solvent, and on the strength of the current. These factors must be chosen arbitrarily.

4. The anesthetics may be compared by either of the following methods: (*a*) by the time in which a given concentration produces paralysis, or (*b*) by the concentration that produces paralysis in a given time.

The first of these comparisons was used by Gros, Symes and Veley and Closson. It can be applied only with precautions. It is true that within certain limits (fifteen to 100 minutes), the paralysis occurs the more rapidly, the more concentrated the solution; but the relation is not a simple quantitative one, and may conceivably vary for different substances.

The second comparison was used by Laewen (one hour), and by Zorn (one-half hour). It also is not quite simple when two different substances are to be compared; for the ratio may be different for paralysis occurring in five minutes and paralysis occurring in two hours. In fact, however, my results indicate that the ratios appear to be practically the same for different periods.

Technic.—The methods of every experimenter so far have differed so much that their results cannot be compared quantitatively. There was, consequently, no advantage in adhering to the arbitrary details of others. Nor was there any advantage in complicating the work by aiming at a theoretical degree of exactness of which the method was inherently incapable, and which would not have much practical importance. In this spirit, the following procedure was evolved, generally dictated by convenience:

The muscle-nerve preparations were made so as to include the lower end of the leg from the knee down,

and the entire sciatic nerve from the knee to the spinal cord with a bit of bone attached.

The preparations were laid in M/8 NaCl (0.73 per cent.) made with tap water. In making the tests, the entire nerve was immersed in the anesthetic solution, contained in a little trough cut into a block of paraffin. Each trough held about 1 c.c. of solution. The excitability was tested with the platinum electrodes of a Harvard induction coil, activated by a current of about 4 volts, with the secondary at 12 cm. This stimulation is, of course, considerably above the threshold.

The stimuli were applied at the distal end of the nerve, that is, within 1 cm. of the spinal origin. Generally, when the block was completed at this point, response could be obtained by moving the electrodes half down the nerve; but this was disregarded.

The experiments were made at temperatures between 18 and 21 C. All the frogs (*Rana pipiens*) came in the same shipment.

The anesthetics were dissolved in M/8 NaCl (0.73 per cent.); except that the concentrated solutions of antipyrin (4 per cent.), KCl (1 per cent.), $NaHCO_3$ (1 per cent.), and Na_2HPO_4 (M/8) were made with water and diluted with saline.

The concentrations of the anesthetics were in geometric ratio (¼, ½, 1, 2, 4, etc.).

II. RELATIVE EFFICIENCY OF THE ANESTHETICS ON MOTOR FIBERS

The mean of the observations is shown in Table 1.

In Table 2, the same data are arranged in another, perhaps more convenient, manner. In the right half of the table, the "efficiency ratio" has been calculated, cocain being taken as the unit. For instance, paralysis is produced in five to ten minutes by cocain, 1 per cent.; novocain, 1 per cent.; antipyrin, 4 per cent.; and KCl, ½ per cent. The novocain would therefore be as efficient as cocain (efficiency ratio = 1); antipyrin is one fourth as efficient (efficiency ratio = ¼); KCl is twice as efficient (efficiency ratio = 2), etc.

The efficiency ratios are shown for each period of time in which the paralyses were observed. The varia-

TABLE 1. TIME OF PARALYSIS OF SCIATIC NERVE

	Per Cent.								
	4	2	1	1/2	1/4	1/8	1/16	1/32	1/64
	Minutes	Minutes	Minutes	Minutes	Minutes	Minutes	Minutes	Minutes	Minutes
Cocain hydrochlorid	....	.....	5 ×10*	×5	20 - 30	30 - 45	45 - 60	90 ×	
Novocain hydrochlorid	...	.....	5 ×10	10 × 15	15 - 20	15 - 25	65 - 90	90 ×	
Tropacocain hydrochlorid	....	.....	10 × 15	15 - 20	15 - 20	20 - 30	35 - 45	120 ×	
Alypin hydrochlorid	....	.....	15 - 20	15 - 20	30 × 45	30 - 45	90 - 120	120 ×	
Antipyrin	× 12	25 - 35	35 × 40						
Quinin urea hydrochlorid	....	.....	45 × 65	45 × 65	45 - 65	60 ×			
KCl	....	.....	×4	5 × 10	30 × 45	15 × 65	90 ×	45 - 65	90 ×
NaCl M/8 = 1	....	.....	110 ×						
$NaHCO_3$	...	.....	×5						
Na_2HPO_4 M/8 = 1	...	.....	85 ×						

* In this and all other tables, the numbers to the left of the × denote the time (minutes) when there was no paralysis; and the number to the right of the × denotes the time (minutes) when paralysis was completed.

TABLE 2. CONCENTRATIONS PRODUCING MOTOR PARALYSIS IN A GIVEN TIME

	Time of Motor Paralysis									Ratio of Efficiency (Paralytic Concentrations) to Cocain = 1					
Minutes	0-5	5-10	10-15	15-20	20-30	30-45	45-65	65-90	>90	0-10	10-20	20-45	45-90	>90	Mean
Drugs:	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.						
Cocain hydrochlor		1 and 1/2			1/4	1/8	1/16		1/32	1	1	1	1	1	1
Novocain hydrochlor		1	1/2	1/4	1/8			1/16	1/32	1/2-1	1	2	1	1	1
Tropacocain hydrochlor			1	1/2 and 1/4	1/8	1/16			1/32	1/4	1/2-1	1 2	1	1	1
Alypin hydrochlor				1 and 1/2		1/4 and 1/8			1/16 and 1/32		1/2	1/8	1/2 1	1/2 1	2 4
Antipyrin		4			2	1				1/4					1/8
Quinin urea hydrochlor							1 to 1/4		1/8				1/1 1/16	1 4	1/5
KCl	1	1/2				1/4 and 1/8			1/16-1 64	1	1	1	1/2	1/2 2	1
NaCl M/8									M/8						8
$NaHCO_3$									1						4
Na_2HPO_4									M/8						

tions are so irregular, that they are probably accidental errors. The mean of all the observations is therefore given in the last column.

In brief, for the motor fibers of the frog's sciatic nerve immersed in the solutions, the relative paralytic efficiency (efficiency ratio) averages:

Cocain hydrochlorid Novocain hydrochlorid Tropacocain hydrochlorid KCl	1
Alypin hydrochlorid	$\frac{3}{4}$
Quinin urea hydrochlorid	$\frac{1}{5}$
Antipyrin	$\frac{1}{8}$

In experimental work, for paralysis in thirty to forty-five minutes, the following were used as practically equivalent minimal effective concentrations:

	Per Cent.
Cocain hydrochlorid Novocain hydrochlorid Tropacocain hydrochlorid	$\frac{1}{8}$
Quinin urea hydrochlorid	1
KCl	$\frac{1}{4}$

M/8 NaCl, $NaHCO_3$ and Na_2HPO_4 do not paralyze in the time of observation.

Results of other investigators. The data on the efficiency of the anesthetics on motor fibers may be compared by tabulating the concentrations that produce complete block in one-half hour. These are shown in Table 3. The actual data vary widely, as was to be expected. The ratios in terms of cocain, however, agree quite well.

III. EFFECTS OF ALKALI

Gros,[3] 1910–1912, found that the addition of alkalis to the hydrochlorids of the anesthetic bases increases their efficiency very materially. He explains this by the liberation of the free bases, which penetrate the nerves much more readily than the water-soluble salts. He found further that the efficiency of the various salts of novocain is proportional to their hydrolysis: i. e., to the degree in which the free base is dissociated.

At first he added the amount of sodium bicarbonate calculated to bind the chlorid. He found, however,

TABLE 3. COMPARISON OF INVESTIGATORS

Minimal concentrations (per cent.) of anesthetics that block the motor fibers within one-half hour

	Symes and Veley	Gros*	Zorn (Winter)	Zorn (Summer)	Closson	Sollmann	Ratio of Eficiency on the Basis of Cocain = 1					
							Symes and Veley	Zorn (Winter)	Zorn (Summer)	Closson	Sollmann	
											Thirty Minutes	Mean of All Periods
Cocain hydrochlorid	1/15	>1/3	1/2	1	1/10	1/4	1	1	1	1	1	1
Novocain hydrochlorid		1	1/2	3/4	2/10	1/8		1	1 1/3	1/2	2	1
Tropacocain hydrochlorid			1/2		1/10	1/8		1		1	2	1
Alpin hydrochlorid		>1/3	3/4		1/10	1/3		2/3		1	1/2	3/4
Beta eucain hydrochlorid		>1/3	3 1/4		>1/10			1/6		<1		
Stovain hydrochlorid	<1/40	>1/4	1/2		<1/10		>3	1		<1		
Quinin urea hydrochlorid					1/3	>1				1/3	<1/4	1/8
Antipyrin				2 1/4		2			1/2		1/8	1/5
KCl				1/2		1/4			2		1	1
KNO_3				1/3					3			
K_2SO_4				1/4	>1/5				4	<1/2		

* Arch. Exp. Path. Pharm., 1910, **63**, 80.

that an excess of alkali gave still greater efficiency, and therefore employed isotonic sodium bicarbonate or phosphate.

It is evident that these results cannot be transferred directly to the clinic. Granting that the free bases penetrate more readily than the salts, it must be remembered that in the clinical application, the anesthetics do not usually reach the normal fibers as pure salts, but that they are partly converted into carbonates by the alkalinity of the tissues. The importance of the alkalization of the solution would therefore presumably be smaller. This again is a question for investigation by other methods, and may be left for future consideration. For the present, it is important to check Gros' results.

It was necessary, in the first place, to make certain that the akalis, sodium bicarbonate and sodium phosphate do not have an anesthetic action. Table 1 shows that they are not in themselves anesthetic.

The following series of experiments were then made:

The solutions of the anesthetic hydrochlorids were mixed with 1 per cent. $NaHCO_3$, or with M/8 Na_2HPO_4, so that the solutions contained:

Per Cent of Anesthetic Hydrochlorid	Per Cent of $NaHCO_3$ or Ratio of M/8 Na_2HPO_4
1/8	1/2
1/16	3/4
1/32	7/8
1/64	15/16

In the case of cocain, another set of experiments was made by adding $NaHCO_3$ in the concentration of one-fourth of the percentage of the cocain hydrochlorid, i. e., just about the amount required to bind the acid.

The detailed results are shown in Table 4. The three series gave practically identical results, so that they may be grouped together, as in Table 5.

The ratio of efficiency, as judged by the concentration required to produce paralysis in a given time, is increased as shown in the last column of Table 5, namely: eight times for cocain and novocain, four

TABLE 4. EFFECTS OF ALKALI ON MOTOR NERVE PARALYSIS

(Time of Paralysis in Minutes)

	Per Cent. of Anesthetic			
	1/8	1/16	1/32	1/64
Cocain with bicarbonate (just sufficient)	5 × 10	5 × 10	15 × 20	45 × 60
Cocain with bicarbonate (excess of bicarbonate)	5 × 10	10 × 15	20 × 30	20 × 30
Cocain with phosphate (excess of phosphate)	10 × 15	× 5	15 × 20	
Novocain with bicarbonate (excess of bicarbonate)	5 × 10	5 × 10	20 × 30	20 × 30
Novocain with phosphate (excess of phosphate)	5×10	10×15	10×15	
Tropacocain with bicarbonate (excess of bicarbonate)	× 5	5 × 10	30 × 45	60 × 75
Tropacocain with phosphate (excess of phosphate)	15 × 20	30 45	45 × 65	
Alypin with bicarbonate (excess of bicarbonate)	15 × 20	20 × 30	30 × 45	45 × 60
Alypin with phosphate (excess of phosphate)	15 × 20	30 × 45	35 × 45	

times for tropacocain, and six times for alypin. It is doubtful whether the differences are significant; it would probably be correct to say that all are potentiated four to eight times by the addition of alkali. This is in great contrast to potassium, which is not potentiated at all by alkali:

	¼ Per Cent.	⅛ Per Cent.
Paralysis occurs in KCl:		
Without alkali	30 × 45 minutes	15 × 65 minutes
With alkali	65 × 90 minutes	90 ×

Comparison with the Results of Gros.—It is not possible to make quantitative comparisons with Gros' results, since he left the nerve in the solutions of the hydrochlorid for one and one-half to fourteen hours—which creates conditions quite different from those in my experiments. Qualitatively, my results confirm his conclusions, that the anesthetic bases are markedly more effective on the excised nerve than are their hydrochlorids.

IV. EFFECT OF EPINEPHRIN

H. Braun,[9] 1903, found that epinephrin increases very greatly the efficiency and duration of anesthesia in wheals, and in the clinical use. He attributes this to the vasoconstriction, preventing the absorptive removal of the anesthetic.

9. Braun, H.: Arch. f. klin. Chir., 1903, **69**, 541; München. med. Wchnschr., 1903, No. 8, p. 352.

TABLE 5.—EFFECTS OF ALKALI. COMPARISON OF MEAN RESULTS OF HYDROCHLORIDS AND BASE

(Time of Paralysis in Minutes)

	Per Cent. of Anesthetic							Efficiency Ratio*
	1	1/2	1/4	1/8	1/16	1/32	1/64	
Cocain hydrochlorid	5×10	×5	20×30	30×45	45×60	×90		
Cocain base				5×10	5×10	20×30	30×45	8
Novocain hydrochlorid	5×10	10×15	15×20	15×25	65×90	90×		
Novocain base				5×10	5×10	15×20	20×30	8
Tropacocain hydrochlorid	10×15	15×20	15×20	20×30	35×45	120×		
Tropacocain base				5×15	10×30	30×65	60×75	2 to 8, mean 4
Alypin hydrochlorid	15×20	15×20	30×45	30×45	90×120	120×		
Alypin base				15×20	20×45	30×45	45×60	4 to 8, mean 6

* As judged by the concentration required to produce paralysis in a given time, the base is x times as efficient as the hydrochlorid.

Esch,[10] 1910, working under Kochmann, reports a series of experiments on the motor functions of the isolated mammalian sciatic nerve. He believes that these establish that there is also a more direct potentiation of cocain, novocain and alypin by epinephrin; tropacocain was not potentiated.

The results, however, are not very striking and not in themselves convincing. However, further work seemed desirable, especially since cocain and epinephrin are synergistic in some other respects. The results are shown in Table 6.

Evidently, the paralytic action of cocain and novocain is not increased directly by epinephrin.

TABLE 6.—EFFECT OF EPINEPHRIN

	Time in Which Paralysis Occurs After Immersion in			
	Cocain Hydrochlorid Concentration		Novocain Hydrochlorid Concentration	
	1/16 Per Cent. Minutes	1/32 Per Cent. Minutes	1/16 Per Cent. Minutes	1/32 Per Cent. Minutes
Without epinephrin	45-60	>90	65-90	>90
With epinephrin	65-90	>90	65-90	>90

The solutions contained epinephrin 1:20,000 for 1/16 per cent. of the anesthetic; 1:15,000 for 1/32 per cent. of the anesthetic.

V. MIXTURES OF COCAIN WITH NOVOCAIN OR QUININ-UREA HYDROCHLORID

The question of the possible synergism of the anesthetics would have both practical and theoretical importance. It has so far been studied mainly by other methods.

When two anesthetics are combined, the effects may be the simple sum of their separate effects (simple summation); or they may be greater (potentiation); or smaller (negative potentiation).

When investigating this question, it is simplest to mix the anesthetics in the proportion of their minimal effective doses. For instance, the minimal effective doses to produce paralysis in thirty to forty-five minutes is, for cocain or novocain hydrochlorids, 1/8 per cent.; for quinin-urea hydrochlorid, it is 1 per cent.

10. Esch, P.: Arch. f. exper. Path. u. Pharmakol., 1910, **64**, 84.

If solutions of this strength are mixed with each other in any proportion, they will paralyze in thirty to forty-five minutes if there is simple summation. If paralysis occurs in a shorter time, there is potentiation.

The degree of potentiation may be expressed in two ways; either (*a*) as the ratio in which the minimal effective doses of the constituents must be increased to produce paralysis in the same time as the mixture, which we may call the "Velocity Ratio;" or (*b*) as the ratio to which the mixture must be diluted to paralyze in the standard time of thirty to forty-five minutes, which we may call the "Dilution Ratio."

For instance, assume that paralysis is produced in thirty to forty-five minutes by anesthetic *X* in the concentration of ⅛ per cent.; and by anesthetic *Y* in the concentration of ½ per cent. These are their minimal effective doses. Let us assume a mixture *Z* of equal parts of these solutions. This would contain 1/16 per cent. of *X* and ¼ per cent. of *Y*. If there is simple summation, it should paralyze in thirty to forty-five minutes.

Let us assume that it paralyzes in ten to fifteen minutes. Let us assume that the concentration of *X* or *Y* necessary to paralyze in ten to fifteen minutes is four times that which paralyzes in thirty to forty-five minutes (i. e., ½ per cent. for *X* or 2 per cent. for *Y*). The potentiation by the velocity ratio is therefore 4.

We may also dilute *Z* until it paralyzes in thirty to forty-five minutes. Let us assume that this takes 3 parts of normal saline to 1 part of *Z*. This would show that *Z* is four times as strong as *X* or *Y*. The potentiation by the dilution ratio would also be 4.

In practice, the velocity ratio and the dilution ratio are generally not identical, and the velocity ratio may also differ according to whether one figures it from *X* or *Y*. The conclusions, in other words, are only approximate, i. e., within the range specified for each case, but they are sufficient for most practical purposes.

The results are shown in Tables 7 and 8.

Neither novocain nor quinin urea show any potentiation with cocain. Indeed, there is not even perfect summation, but this is probably within the experimental error.

VI. MIXTURES OF ANESTHETICS AND POTASSIUM

Hoffmann and Kochmann,[5] 1912, found that mixtures of potassium and novocain give a well marked potentiation. Their results with the sciatic nerve indicate a potentiation of 2 2/3 by the dilution ratio, between novocain and potassium. Their later work was done with the wheal method, and will be considered in another paper.

TABLE 7.—MIXTURES OF COCAIN WITH NOVOCAIN (HYDROCHLORID)

Per Cent. of Cocain	Per Cent. of Novocain	Total Per Cent.	Time of Paralysis	Per Cent. of Unmixed Anesthetics That Would Paralyze in Same Time	Efficiency Ratio by Velocity
1/16	1/16	1/8	65–90	1/16	1/2
1/32	1/32	1/16	>90	1/32	1/2
1/64	1/64	1/32	>90		

TABLE 8. MIXTURES OF COCAIN WITH QUININ UREA (HYDROCHLORID)

Percentage of		Total Minimal Effective Concentration,* (30-45 Min.)	Time of Paralysis	Minimal Effective Concentration of Single Anesthetics Required to Paralyze in the Same Time		Efficiency Ratio by Velocity
Cocain	Quinin Urea		Minutes	Cocain	Quinin Urea	
1/16	1/2	1	45–65	1/2	1/4–1	1/4–1
1/32	1/4	1/2	65–90	1/2–1/4	1/8–1/4	1/4–1
1/64	1/8	1/4	>90	1/4	1/8	1/2–1

* The minimal effective concentration of cocain is 1/8 per cent.; of quinin urea, 1 per cent. A solution containing 1/32 per cent. of cocain and 1/4 per cent. of quinin urea would contain 1/4 minimal effective concentration of each, or a total of 1/2 minimal effective concentration.

With the object of checking and expanding these data, series of experiments were made with mixtures of the individual anesthetics and potassium chlorid, by using the two anesthetics in the ratio of their "minimal effective concentration" thirty to forty-five minutes, i. e., the concentrations producing complete block in thirty to forty-five minutes, as stated below. The solutions were mixed so as to contain various proportions of the two ingredients, but with the minimal effective concentration unaltered; and these mixtures were then diluted to various degrees; the time of block being

determined in each case. This was compared with the concentration of either agent alone that would give block in the same time.

For instance:

Cocain, ⅛ per cent., paralyzes in thirty to forty-five minutes
KCl, ¼ per cent., paralyzes in thirty to forty-five minutes

A mixture of equal parts of these (therefore having the same minimal effective concentration = 1) paralyzes in five to ten minutes.

To produce paralysis in this time, would require:

Of cocain alone, ½ to 1 per cent., i. e., four to eight times the total minimal effective concentration of the mixture, or a potentiation of four to eight times.

Of potassium chlorid alone, ½ per cent., i. e., two to four times the total minimal effective concentration of the mixture, or a potentiation of two to four times. The potentiation acidity to velocity is therefore two to eight.

The mixture was diluted with normal saline so as to contain the following fractions of the original concentrations; and then produced paralysis in the following time:

Dilution to ½ = 20 × 25 minutes
Dilution to ¼ = 45 × 65 minutes
Dilution to ⅛ = 90 × minutes
Dilution to 1/16 = 90 × minutes

Consequently, the mixture had to be diluted two to four times to produce paralysis in thirty to forty-five minutes, i. e., the potentiation acidity to dilution was two to four.

Table 9 contains the detailed data, and Table 10 the potentiation ratios deduced from them.

The results show quite marked potentiation, the efficiency of the mixtures being up to eight times as high as that of the unmixed components; thus confirming Hoffmann and Kochmann.[5] The velocity ratios give higher figures than the dilution ratios. The most favorable results are observed if the minimal effective concentration ratio of potassium equals or exceeds that of the synergistic anesthetic.

VII. MIXTURES OF ANESTHETICS WITH BICARBONATE AND POTASSIUM

From a practical standpoint, it appeared interesting to determine the results of combining the synergism of potassium and alkalization. The results are shown in Table 11.

The bicarbonate increases the efficiency of these cocain mixtures by two to four times; whilst it increases the efficiency of the cocain itself eight times. Since only a fourth of the mixture consists of cocain, this would correspond exactly to simple summation of the alkali and potassium, without any additional potentiation.

With the novocain mixtures, the results are not quite so favorable; but they probably fall within the experimental error.

It appears therefore that the beneficial effects of alkali on cocain are preserved but not increased in the cocain-potassium mixture; and this probably holds also for novocain.

TABLE 9.—MIXTURES OF POTASSIUM AND ANESTHETICS ON SCIATIC MOTOR FIBERS OF THE FROG

Ratio of Minimal Effective Concentration of Synergist: KCl = Total	Per Cent. of Cocain, Novocain, Tropacocain (Hydrochlorid) (Minimal Effective Concentration = 1/8 per Cent.)	Per Cent. of KCl (Minimal Effective Concentration = 1/4 per Cent.)	Time of Paralysis in Mixtures: Cocain KCl	Novocain KCl	Tropacocain KCl	Quinin Urea KCl	Per Cent. of Quinin Urea Hydrochlorid (Minimal Effective Concentration = 1 per Cent.)
15: 1 = 1	15/128	1/64	30 - 40				
7: 1 = 1	7/64	1/32	20 - 30	30×45	45×65		
3: 1 = 1	3/32	1/16	20 - 30	15×25	20 - 30	0×5	3/4
1: 1 = 1	1/16	1/8	5 - 10	10×15	10 - 15	0 - 5	1/2
1: 3 = 1	1/32	3/16	10×15 15 - 20	90× 10×15	5×10	20 - 30	1/4
1: 7 = 1	1/64	7/32	×5	90×	10×15		
1:15 = 1	1/128	15/64	20×30				
1: 1 = 1/2	1/32	1/16	20 - 25	60×95	65×90	65 - 90	1/4
1: 1 = 1/4	1/64	1/32	45×65	90×	90×	90×	1/8
1: 1 = 1/8	1/128	1/64	90×	90×	90×	90×	1/16
1: 1 = 1/16	1/256	1/128	90×	45×65	90×	90×	1/32
1: 3 = 1/2	1/64	3/32	30×45 30 - 45	15×20 90×	45×65		
1: 3 = 1/4	1/128	3/64	45 - 65 45 - 65	65 - 90 90×	90×		
1: 3 = 1/8	1/256	3/128	45 - 65 90×	90× 90	65×90		
1: 3 = 1/16	1/512	3/256	45×65 90×				
1: 7 = 1/2	1/128	7/64	×5	90×	65 - 90		
1: 7 = 1/4	1/256	7/128	90×	90×	90×		
1: 7 = 1/8	1/512	7/256	90×	45×60	65 - 90		
1: 7 = 1/16	1/1024	7/512	90×				
1:15 = 1/2	1/256	15/128	0 - 5				
1:15 = 1/4	1/512	15/256	30×45				
1:15 = 1/8	1/1024	15/512	75×90				

TABLE 10. POTENTIATION RATIOS OF POTASSIUM AND ANESTHETICS

Ratio of Anesthetic (of Percentage Stated Under Each Anesthetic)	KCl (¼ per Cent.)	Potentiation With: Cocain Hydrochlorid (⅛ per Cent.) by: Velocity	Cocain Hydrochlorid (⅛ per Cent.) by: Dilution	Novocain Hydrochlorid (⅛ per Cent.) by: Velocity	Novocain Hydrochlorid (⅛ per Cent.) by: Dilution	Tropacocain Hydrochlorid (⅛ per Cent.) by: Velocity	Tropacocain Hydrochlorid (⅛ per Cent.) by: Dilution	Quinin Urea Hydrochlorid (1 per Cent.) by: Velocity	Quinin Urea Hydrochlorid (1 per Cent.) by: Dilution
15	1	1				½=1			
7	1	1–2		1		1		4-x	
3	1	1–2		1–2		2–8	0	4-x	0
1	1	2–8	2 4	4	0	2–8	0	4-x	
1	3	1–4	2	¼–4	2	2–8	0		
1	7	4–8	2	¼	0				
1	15	1–2	4						

TABLE 11.—MIXTURES OF COCAIN AND NOVOCAIN WITH POTASSIUM AND BICARBONATE

The anesthetics are adjusted so as to make the ratio of the minimal effective concentration of cocain or novocain hydrochlorid (⅛ per cent.) to that of potassium chlorid (¼ per cent.) as 1:3.

Cocain Hydrochlorid Per Cent.	Potassium Chlorid Per Cent.	Total Minimal Effective Concentration	Time of Paralysis Without Alkali	Time of Paralysis in Presence of Alkali	Ratio in Which the Mixtures Are Potentiated by Alkali According to: Velocity	Dilution
1/8	0	1	30-45	5×10		8
0	1/4	1	30-45	65×95		>1
1/32	3/16	1	10-20			
1/64	3/32	1/2	30-45	15×20	4	2
1/128	3/64	1/4	45 - 65	30×45	4	2
1/256	3/128	1/8	65×90	30×45	4	4
1/512	3/256	1/32	65×90	90×		
Novocain Hydrochlorid						
1/8	0	1	15-25	5×10		8
1/32	3/16	1	30-45			
1/64	3/32	1/2	20 - 90	30 - 45		2
1/128	3/64	1/4	65 - 90	90×	1	
1/256	3/128	1/8	90×	90×		
1/512	3/256	1/32	90×	90×		

VIII. CONCLUSIONS

1. Comparisons of the efficiency of local anesthetics must be adapted to the special uses of these agents. A complete review of the more important drug from this standpoint seems desirable. The present paper deals with the motor nerve fibers of the frog, and should not be transferred directly to clinical conditions.

2. With direct application to the motor nerve fibers of the frog, cocain, novocain and tropacocain hydrochlorids and potassium chlorid are about equally effi-

cient. The efficiency of alypin hydrochlorid is about three-fourths, quinin-urea hydrochlorid one-fifth and antipyrin one-eighth of the efficiency of cocain hydrochlorid.

3. Basic cocain, novocain, tropacocain and alypin are four to eight times a effective as the hydrochlorids. The addition of bicarbonate to potassium does not have this effect.

4. The addition of epinephrin does not increase the efficiency of cocain or novocain on the excised nerve.

5. The effects of mixture of cocain with novocain or quinin-urea hydrochlorid do not show any potentiation.

6. The addition of potassium to cocain, novocain, tropacocain and quinin-urea hydrochlorid gives marked potentiation; the efficiency being up to eight times greater than would correspond to simple summation of the effects of the ingredients.

7. The potentiation by potassium holds also for basic cocain. That is, the potentiation by potassium and by alkali are simply additive.

COMPARATIVE SYMPTOMS RESULTING FROM THE USE OF SOLUTIONS OF MERCURIC IODID IN OIL

Oily solutions of mercuric iodid have been used to some extent for intramuscular injections. Special claims of superiority have been made for a proprietary solution of this kind, sold under the name "Biniodol." It appeared interesting to determine whether this special solution really possesses any superiority; or whether a simple oily solution has the same properties. The cooperation of Dr. H. N. Cole, of the Department of Dermatology and Syphilology of the Western Reserve University, cooperating with Cleveland City Hospital, and of Dr. Albert Keidel, of the Johns Hopkins Hospital, made it possible to utilize the "blind test" for this purpose. Each collaborator received three samples, labeled, respectively, 1, 2 and 3; 1 contained biniodol; 2, a 1 per cent. solution of mercuric iodid in oil; 3, a solution made up according to the formula of biniodol, namely, 1 per cent. of mercuric iodid and 2.5 per cent. of guaiacol in oil. All the solutions were sterile. The investigators were not informed which preparation was designated by the respective numbers, but they were asked to use the preparations when intramuscular injections of a 1 per cent. oily solution of mercuric iodid were indicated, and to note what differences, if any, were observed following the use of the different solutions regarding pain, discomfort, induration and any other evidences of effects of the medicaments.

REPORT OF DR. H. N. COLE[1]

At the request of Prof. Torald Sollmann of the Council on Pharmacy and Chemistry of the American Medical Association, we made a comparative study of several oily preparations of red mercuric iodid for intramuscular injections in syphilis.

The information, concerning the preparations submitted to the investigators, was as follows:

1. From the Department of Dermatology and Syphilology of the Western Reserve University and of the Cleveland City Hospital.

"OILY SOLUTION OF RED MERCURIC IODID

"It is desired to ascertain whether there is any difference between three preparations, each containing 1 per cent. of mercuric iodid, as to pain, discomfort, induration, etc. The preparations will be labeled "1," "2" and "3." They will be sterile.

"One of these preparations will be a plain solution in oil; another will contain, in addition, 2.5 per cent. of guaiacol; the third will be a proprietary preparation containing the guaiacol.

"It is also desirable to know how the oily solution compares with the plain watery solution; but this is of secondary importance."

The preparations all had the same appearance. The patients were taken indiscriminately, and we attempted to keep them on the injections as long as possible, in order to compare symptoms. Owing, however, to discharge from hospital, symptoms of mercury intoxication, etc., we were unable in all cases to give a thorough trial with each preparation.

In all, eleven patients were treated and seventy-one injections given—by which time our experimental supply was exhausted.

In each case the drug was given intramuscularly in the buttocks and the patients carefully observed for subjective symptoms of pain and for objective symptoms of swelling, induration, abscess formation, etc. The details are given in Table 1.

As will be noted, in several of the cases the patients were more or less confused and gave rather indefinite and conflicting answers. In attempting to compare the results from the different drugs, by careful tabulation one finds that symptoms were more marked with the respective sample as follows:

Preparation 1 was worse than Preparations 2 or 3 in six cases.

Preparation 2 was worse than Preparation 1 in two cases.

Preparation 2 was worse than Preparation 3 in five cases.

Preparation 3 was worse than Preparations 2 or 1 in one case.

The criticism may be raised that the number of cases and of injections is too small to permit the drawing of any just conclusions. Even should we grant it, the statistics certainly do not prove any marked superiority of any one of the preparations over the others. We wish to thank Dr. Sollmann for advising and directing us in this work, and Drs. Bailey, Bernstein, Markus and Reycraft for assistance in carrying it out.

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REPORT OF DR. ALBERT KEIDEL[2].

Twenty cases were chosen at random from the syphilitic patients attending the clinic. They were given intramuscular injections of the three solutions, in amounts varying from 1 to 2 c.c., at intervals (in most instances) of two days. The injections were invariably made into the gluteal muscles, at depths of from 2 to 2½ inches, and ordinary care exercised to preserve asepsis. After injection the patient was allowed to depart, and the result was recorded at the succeeding visit. The result was determined from the patient's statement and our examination. Some patients received injections of only one solution; some were treated with first one and later with another, and one patient received all three at different times. The solutions were never mixed for a single injection, of course.

TABLE 2.—REACTIONS IN TWENTY CASES REPORTED BY DR. KEIDEL

Preparation	Reactions				Number of Injections
	Severe	Mild	None	Undetermined	
1	13	14	4	8	39
2	5	15	16	5	41
3	7	25	3	2	37
					117

The solutions are understood to contain a 1 per cent. solution of red mercuric iodid in oil, two of them containing in addition 2.5 per cent. of guaiacol, one of these being a proprietary preparation. The solutions are designated as Preparations 1, 2 and 3, respectively, corresponding to the numbers on the labels of the bottles in which they were originally received. The local reactions are recorded as "severe" (S), "mild" (M), "none" (0) and "Undetermined" (U). By "severe" is meant very severe pain lasting for from several hours to several days; by "mild" is meant slight pain or numbness for several hours, or less than an hour; "none" indicates that there was no local reaction, and "undetermined," that the patient has failed to return after the last injection.

In Table 3 all the details of the investigation are recorded. Under "Local Reaction," the letters represent the type of reaction after each injection, in the order in which they were given; when two solutions were used in the same case, the

2. From the Department of Syphilis of the Johns Hopkins Hospital and Dispensary.

TABLE 3.—DETAILS OF INVESTIGATION BY DR. KEIDEL

Case	No.	Preparation	Local Reaction	Total Amount Solution Given, C.c.	Duration of Treatment	Effect on Wassermann	Type of Case	Result	General Remarks
1	3	2	O O O	3	6 da.	+	Latent		
2	5	2	M O S M S	5.6	9 da.	+	Gummas.....	Marked improvement	
3	7	1	M M M; others U	9.5	3 mo.	— to +	Latent		
	3	2	U U U						
4	1	2	U	0.75	.....	+	Latent		
5	4	1	S S S M	4.4	9 da.	—	Gummas.....	.....	After 4th injection, developed diarrhea; melena
6	9	1	O O U M S O S M U	9.1	1 mo.	—	Latent		
7	2	3	M M	3.8	2 da.	+	Latent.....	.....	Well tolerated
8	7	2	O O O O M O U	9.6	17 da.	+ to +	Primary.....	Primary healed	
9	4	1	S M M U	5.5	9 da.	+	Gumma.....	Improved	
10	3	3	M S S	3	6 da.	+	Palmar syphilis; tertiary	Markedly improved	
11	7	3	M S M M M M M	10.6	13 da.	+ to +	Latent		
12	3	2	M M O	5.4	14 da.	+	Secondary (papular)	Rash disappearing. ...	Developed toxic erythema on thighs. Cleared up on stopping $HgCl_2$ and under local treatment
	2	1	S M						
13	10	3	M M M M M M M M M M U	12.6	20 da.	+ to +	Secondary (lichen syph.)	Rash not improved....	Small induration following injection of 1.2 c.c.
14	6	2	O O M S M M	7.2	17 da.	+ to +	Old cerebrospinal syphilis	.....	Responded to doses of 1 c.c. with salivation; fever after injection of 1.2 c.c.
	2	1	S M						
15	4	1	S O M S	4.2	7 da.	+ to +	Secondary (condylomas	No improvement	
16	9	3	O M O M M S M S O	10.4	12 da.	+	Secondary (pustular syph.)	Pustules dried up; headache and fever gone	Slight gingivitis following dose of 1.5 c.c.
	2	2	S O						
17	5	1	S S M S U	13.3	18 da.	+ to +	Tertiary; aortitis....	General condition improved	
	2	2	M S						
	2	3	M S						
18	4	2	O O M M	9.5	13 da.	— to +	Latent		
	2	1	M M						
19	2	3	M U	2.5	5 da.	+	Gumma.....	Markedly improved	
20	5	2	M M M M O	9	14 da.	+ to +	Latent.....	Marked general improvement	Small induration following No. 3
	2	3	M S						

letters represent the reactions following the solution opposite which they stand. In the fifth column the plus and minus symbols indicate the Wassermann reaction; plus indicates a completely positive, and minus a completely negative reaction. When there is only one sign, it refers to the reaction at the end of treatment; when there are two, to the reaction before and after. The seventh column shows the clinical result at the end of treatment; when no note is made, it means that there was no change noted. In the eighth column are noted any objective results observed at the time of examinations of the patients.

The injections were made and the result charted by Dr. E. L. Zimmermann, of my staff, under my directions and supervision.

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